UNIVERSITY OF ALBERTA

Synthetic Studies on 2-Amino-2-Deoxy Glycosides

by

Hailong Jiao

A thesis submitted to the Faculty of Graduate Studies and Research

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

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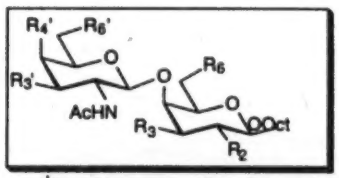
ABSTRACT

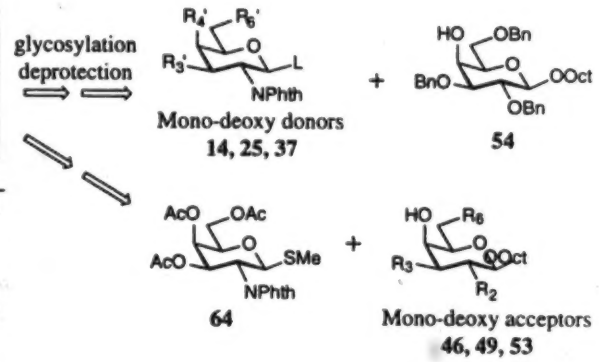
PART I

Pseudomonas aeruginosa is an opportunistic pathogen that employs pili to mediate attachment to host epithelial cells and initiate many infections and disease. Previous studies have shown that the bacterial pili interact with the glycosphingolipid asialo-GM_1 by recognizing the internal disaccharide sequence β-D-GalNAc-(1→4)-β-D-Gal. Two series of analogs of this β-D-GalNAc-(1→4)-β-D-Gal sequence were chemically synthesized for the purpose of studying this binding.

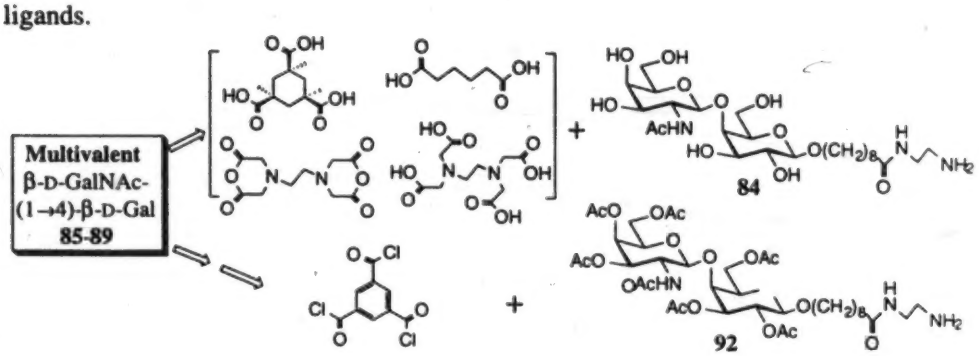
The first series consisted of the six mono-deoxygenated octyl β-D-GalNAc-(1→4)-β-D-Gal analogs **57**, **60**, **63**, **67**, **70** and **74**.



	R_2	R_3	R_6	R_3'	R_4'	R_6'
74	**H**	OH	OH	OH	OH	OH
70	OH	**H**	OH	OH	OH	OH
67	OH	OH	**H**	OH	OH	OH
63	OH	OH	OH	**H**	OH	OH
57	OH	OH	OH	OH	**H**	OH
60	OH	OH	OH	OH	OH	**H**

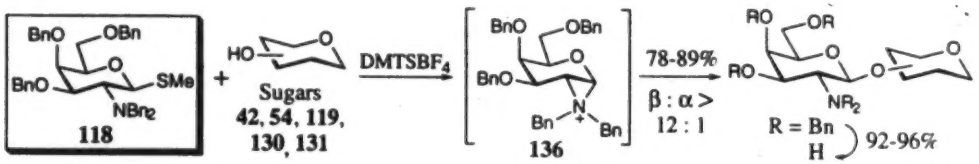


The second series consisted of the five multivalent β-D-GalNAc-(1→4)-β-D-Gal oligomers **85-89**, designed to probe the interaction of pili with multivalent ligands.

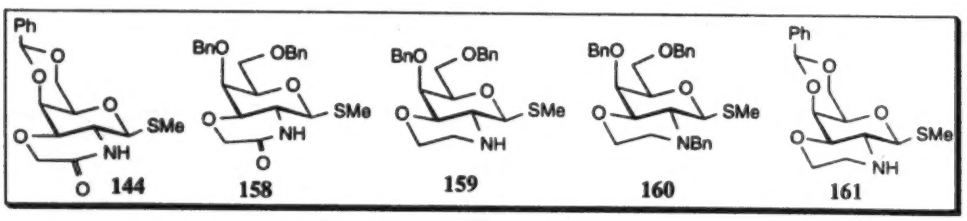


PART II

β-Linked 2-amino-2-deoxy-glycopyranosides are important constituents of proteoglycans, glycoproteins, peptidoglycans and glycolipids which are widely distributed in living organisms and plants. An efficient methodology for the synthesis of this class of compounds was developed by employing the novel glycosyl donor **118**. The β-glycosidic linkage was formed with high β/α-stereoselectivity and in excellent yield for a range of challenging acceptors (**42, 54, 119, 130, 131**). The *N,N*-dibenzyl protecting groups were readily cleaved under the hydrogenolytic conditions commonly used for *O*-debenzylation, facilitating the synthesis of oligosaccharides with free amino-groups.



Also described in this thesis is a model study for 1,2-*cis*-glycosylation by 2-amino sugars utilizing the five new donors **144** and **158-161**, which contained a 3-*O*,2-*N* linker. The glycosylation of various acceptors (alcohol and sugars) with these donors proceeded with high α-stereoselectivity and in good yield.



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LIST OF ABBREVIATIONS

[a]	specific rotation
Ac	acetyl
AIBN	2'2-azobisisobutyronitrile
All	allyl
anal.	analysis
aq.	aqueous
Asn	asparagine
APT	attached proton test
Bn	benzyl
b	broad
BSA	bovine serum albumin
Bu	butyl
Bz	benzoyl
c	concentration (g/100 mL)
calcd	calculated
CAN	cerium (IV) ammonium nitrite
CSA	camphorsulfonic acid
d	doublet or day(s)
DBU	1,8-diazabicyclo[5,4,0]undec-7-ene
DCC	1,3-dicyclohexylcarbodiimide
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIC	diisopropylcarbodiimide
DMAP	4-dimethylaminopyridine
DME	dimethoxyethane
DMF	*N,N*-dimethylformamide

DMTS	dimethyl(methylthio)sulfonium
EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
equiv.	equivalent
Et	ethyl
FAB	fast atom bombardment
Gal	galactopyranoside
GalNAc	2-acetamido-2-deoxy-galactopyranoside
Glc	glucopyranoside
GlcNAc	2-acetamido-2-deoxy-glucopyranoside
HOBt	1-hydroxybenzotriazole
h	hour(s)
Hz	hertz
J	coupling constant
LAH	lithium aluminum hydride
m	multiplet
m/z	mass to charge ratio
Man	mannopyranoside
MCO	8-methoxycarbonyloctyl
Me	methyl
mg	milligram(s)
MHz	megahertz
min	minute(s)
mL	milliliter(s)
mol	mole(s)
mmol	millimole(s)
MS	mass spectrometry or molecular sieves
NeuAc	*N*-acetyl neuraminic acid, sialic acid

NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
Ph	phenyl
Phth	phthaloyl
pMB	*p*-methyoxybenzyl
ppm	parts per million
Pyr	pyridine
q	quartet
R_f	retardation (retention) factor
ROESY	rotating frame nuclear overhauser and exchange spectroscopy
rt	room temperature
s	singlet
Satd	saturated
t	triplet
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
Troc	2,2,2-trichloroethylformate
Ts	*p*-toluenesulfonyl
Xyl	xylose

PART I

Synthesis of Disaccharide Analogs as Potential Inhibitors of Bacterial Adhesion

Chapter 1

Introduction

1.1. Glycobiology Background.

1.1.1. Glycobiology.

Historically, the name carbohydrates was used to describe compounds of the formula $C_n(H_2O)_n$, "carbon-hydrates". Today, the word "carbohydrate" no longer has an exact definition because many carbohydrates are devoid of specific hydroxyl groups or have amino groups or other functionalities. Carbohydrates were traditionally also viewed as energy-storage materials (in the form of monosaccharides and polysaccharides such as starch), structural materials (the polysaccharides cellulose in plants and chitin in the exoskeletons of insects) and primary metabolites that were produced in photosynthesis and were destined for further conversion in nature. In 1923, Avery and Heidelberger demonstrated that the immunoactive, antigenic part of the outer cell wall of the *streptococcus pneumoniae* bacteria was a polysaccharide and not a protein as previously assumed [1]. The discovery that the carbohydrate-covered surfaces can be biologically active ushered in a new era of carbohydrate chemistry and eventually evolved into a new discipline now known as glycobiology. Two decades later, Morgan demonstrated the importance of carbohydrate structures in blood group substances [2]. It thus became clear that carbohydrates played a far wider and much more subtle role in natural processes than earlier believed. Carbohydrates can be found almost everywhere in nature [3, 4].

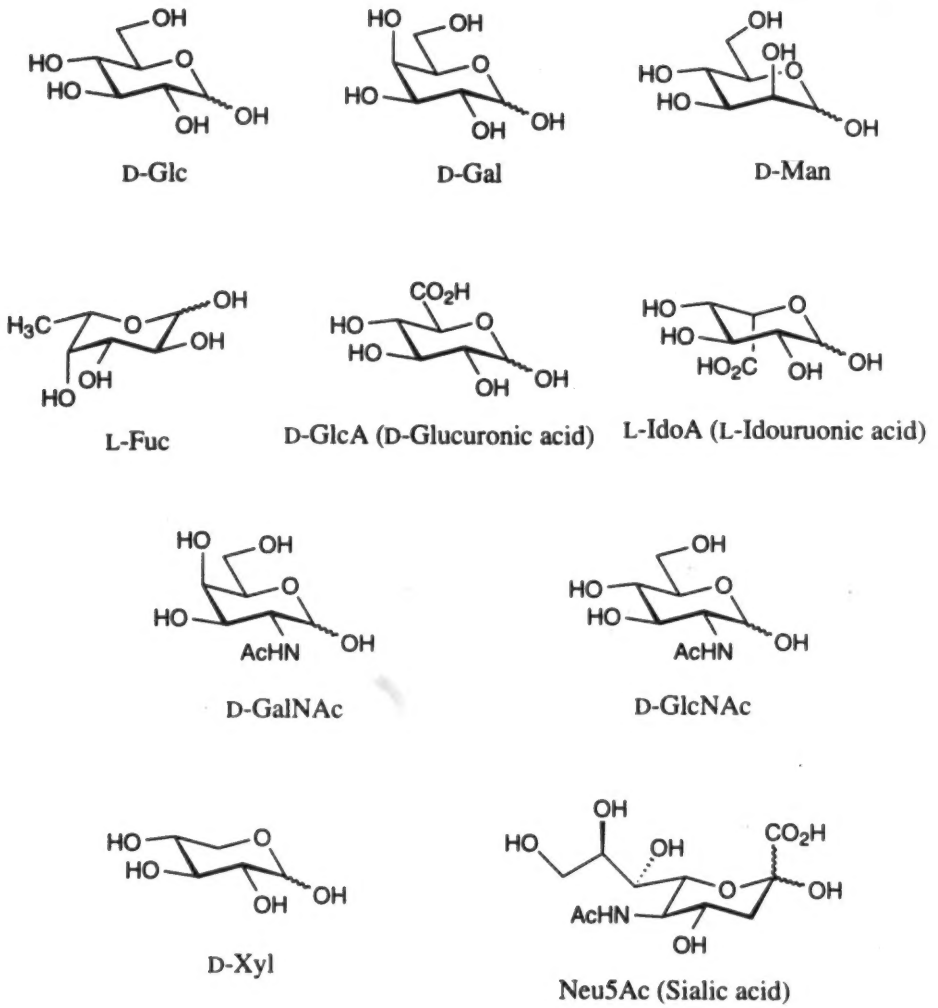
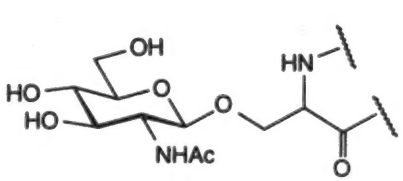


Fig. 1.1: Monosaccharides found in mammalian glycoconjugates.

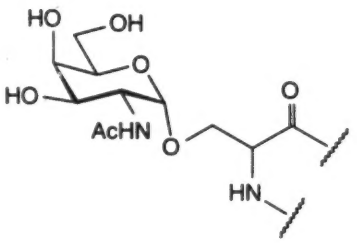
1.1.2. Glycoproteins and Glycolipids.

There are ten monosaccharides [5] (Fig. 1.1) found in mammalian systems which are covalently linked to other types of biomolecules at the anomeric position. These carbohydrate-biomolecule adducts are termed glycoconjugates. The carbohydrate part in glycoconjugates is called the glycan and the non-carbohydrate part is called the aglycon.

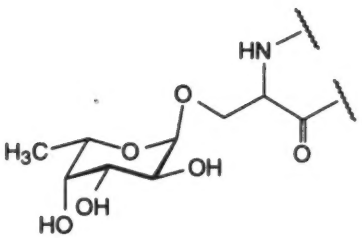
When the aglycon is a protein, the glycoconjugate is called a glycoprotein and when the aglycon is a lipid it is termed a glycolipid. Glycolipids carry only one oligosaccharide per molecule while glycoproteins, through more than one attachment site per molecule, can carry several different glycan chains.



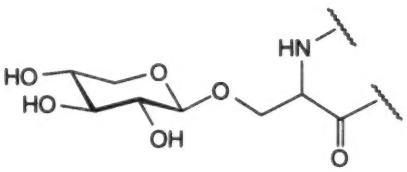
D-GlcNAc-β-Ser linkage



D-GalNAc-α-Ser linkage



L-Fuc-α-Ser linkage



D-Xyl-β-Ser linkage

Fig. 1.2: Examples of *O*-linked glycoprotein linkages.

Most of the oligosaccharides found in glycoproteins are either *N*-linked to the amide nitrogen of asparagine or *O*-linked to the hydroxyl group of serine or threonine [6]. Other linkages such as those to the sulfhydryl group of cysteine [7] are less frequent. *O*-Linked oligosaccharides do not have a common core structure and are not formed on specific amino acid sequences [Fig. 1.2]. On the other hand, *N*-linked oligosaccharides contain a common pentasaccharide core structure that is always linked to the specific amino acid sequence Asn-X-Ser/Thr where X is any amino acid other than proline (Fig. 1.3) [8].

This core pentasaccharide has the sequence α-D-Man-(1→6)-[α-D-Man-(1→3)-β-D-Man-(1→4)-β-D-GlcNAc-(1→4)]-β-D-GlcNAc (Fig. 1.4). The carbohydrates in glycolipids are linked to either ceramide (Fig. 1.5) or phosphorylglycerol (Fig. 1.6).

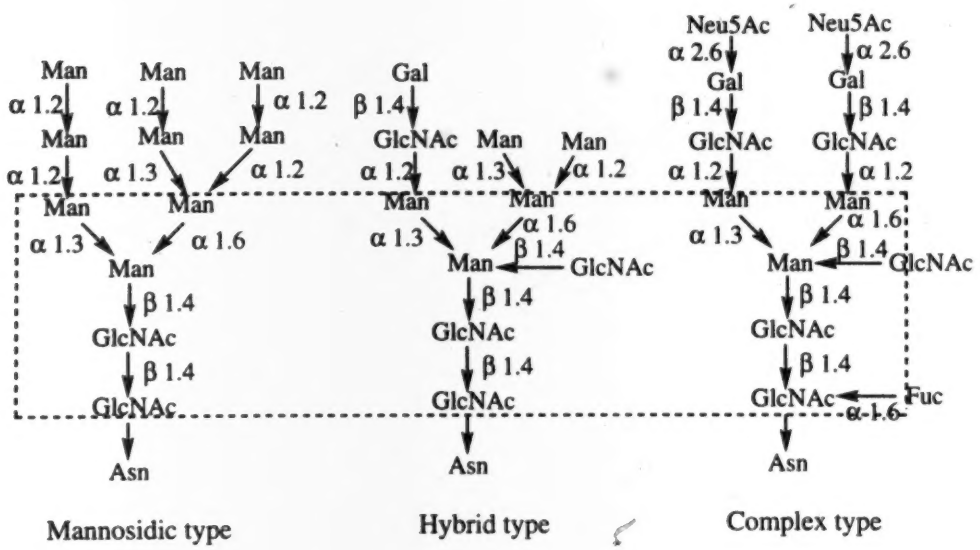


Fig. 1.3: Basic structures of Asn-linked oligosaccharides.

In organisms, oligosaccharides are enzymatically biosynthesized by glycosyltransferases and glycosidases. *N*-Linked glycoproteins are produced co-translationally in the endoplasmatic reticulum followed by trimming and modification by an intricate co-operation between glycosyltransferases and glycosidases. Glycolipids and *O*-linked glycoproteins are formed post-translationally by the action of membrane-bound glycosyltransferases in the endoplasmatic reticulum and Golgi apparatus [9].

1.1.3. The Biological Roles of Carbohydrates.

Biologists generally accept that cells recognize one another through pairs of

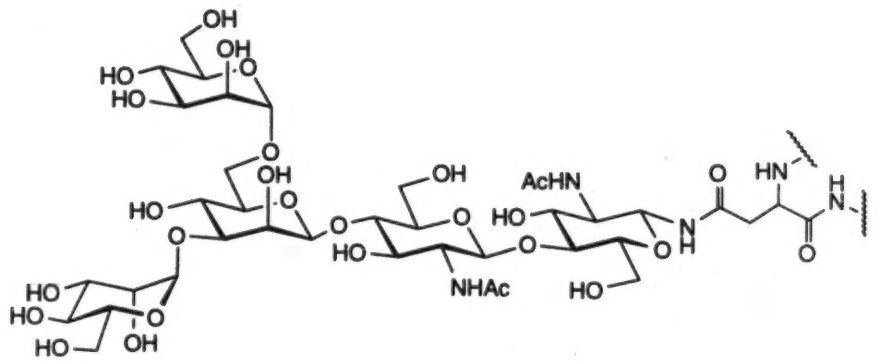
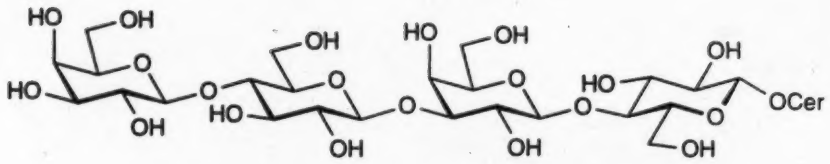


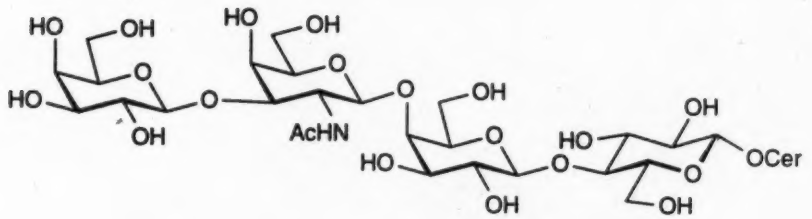
Fig. 1.4: Pentasaccharide core of *N*-linked glycoproteins.

structures on their surfaces such that a structure on one cell carries encoded biological information that the structure on the other cell can decipher. Nature's choice of carbohydrates as information carriers is very clever because oligosaccharides are polyols and a large diversity of structures is possible from a small number of monosaccharides. For example, two identical amino acids can form only one dipeptide, while two monosaccharides can make ten different disaccharides. Because carbohydrates have several connection possibilities they generate additional diversity through branching when three or more monosaccharide units are linked together. Four different amino acids can form only twenty four different tetrapeptides, but four different monosaccharides can make 35,560 different tetrasaccharides [10].

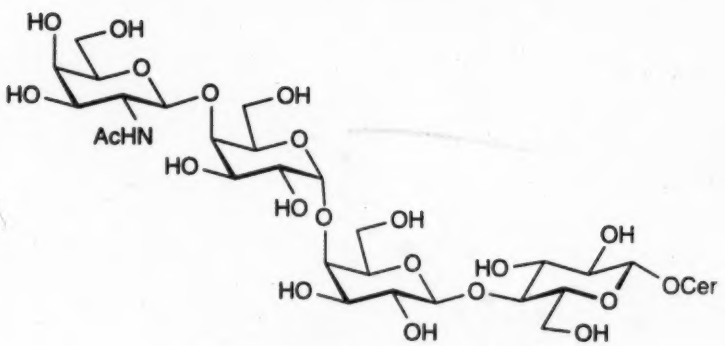
Many biological events involving carbohydrates are known. A review article, entitled "Biological roles of oligosaccharides: all of the theories are correct", cites more than one thousand references [4] describing the biological roles of oligosaccharides including structural, protective and stabilizing roles for polypeptides and proteins, specific receptors for noxious agents, masking and decoys for protection from microorganisms and antibodies, specific receptors for symbiotic functions, on-off and tuning functions for the



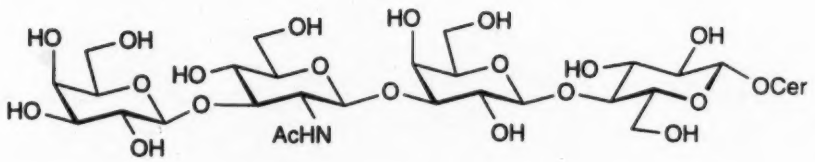
Neolacto-series: Neolactotetraosylceramide



Ganglio-series: Gangliotetraosylceramide



Globo-series: Globotetraosylceramide



Lacto-series: Lactotetraosylceramide

Fig. 1.5: Examples of glycolipid core structures.

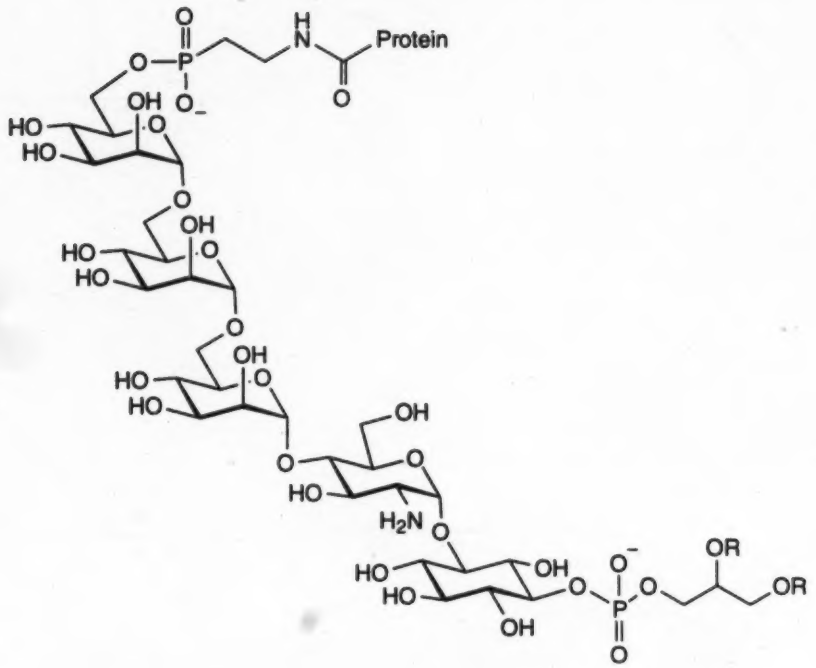


Fig. 1.6: Common core of glycerol-phosphatidyl-inositol (GPI) anchors (R = long chain ester or alkyl groups).

biological activity of proteins, intercellular trafficking functions, regulating the clearance or turnover of proteins and whole cells, hormonal action, cell-cell and cell-matrix recognition and so on.

Among these biological roles of oligosaccharides, the binding of bacteria, viruses, fungi, parasites and toxins to carbohydrate receptors are not beneficial to human [11]. The binding of pathogens to glycoconjugates often causes inflammation, cancer or infection. For example, *Pseudomonas aeruginosa* employs its pili or fimbriae, called adhesins, to bind to glycoconjugates such as the ganglioside asialo-GM_1. Other well-known infectious bacteria which recognize glycoconjugates are *Neisseria gonorrhoeae* (causing gonorrea), *Neisseria meningitidis, Dichelobacter nodosus, Moraxella bovis,*

Vibrio cholerae, and enterotoxigenic *Escherichia coli* [12]. Influenza and Sendai virus, cholera toxin, shiga toxin and verotoxin are examples of viruses and toxins that bind to oligosaccharides .

1.2. Biochemistry Background.

1.2.1. Bacteria.

Bacteria are a type of prokaryote. For almost a century bacteria have been classified as Gram-negative or Gram-positive. Gram-negative and Gram-positive bacteria are distinguished according to whether or not they take up Gram stain (a procedure developed in 1884 by Christian Gram in which heat-fixed cells are successively treated with the dye crystal violet and iodine and then destained with either ethanol or acetone) [13]. This empirical classification is due to differences in composition and construction of the cell envelopes. Gram-negative bacteria have a thick cell wall mainly composed of peptidoglycans (Fig. 1.7), polysaccharide, and teichoic acid [14].

Gram-positive bacteria have a thin peptidoglycan wall, covered by an outer membrane. A unique biopolymer, termed lipopolysaccharide (LPS), is anchored to the outer membrane. This LPS determines the antigenicity, toxicity and invasiveness of the bacteria. Because of its toxic properties it is also referred to as endotoxin. The LPS of a Gram-negative bacterium consists of three parts: lipid A, a core region and the *O*-antigen. The composition of the core region in Gram-negative bacteria is relatively constant, but the *O*-antigenic part is unique. Fig. 1.8 shows a schematic representation of the envelope of a Gram-negative bacteria [15].

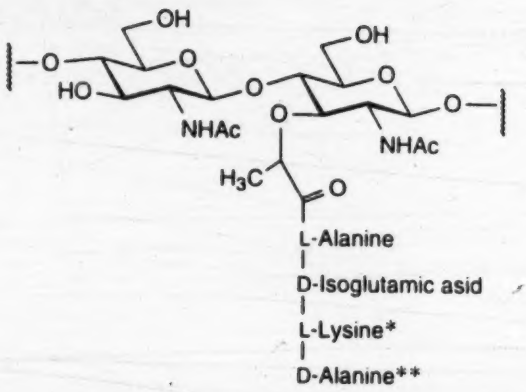


Fig. 1.7: Chemical structure of a peptidoglycan. Cross-links are formed by tetrapetide chains between the amino group of the lysine (*) on one chain and the C-terminal carboxyl group of the another (**) on another tetrapeptide.

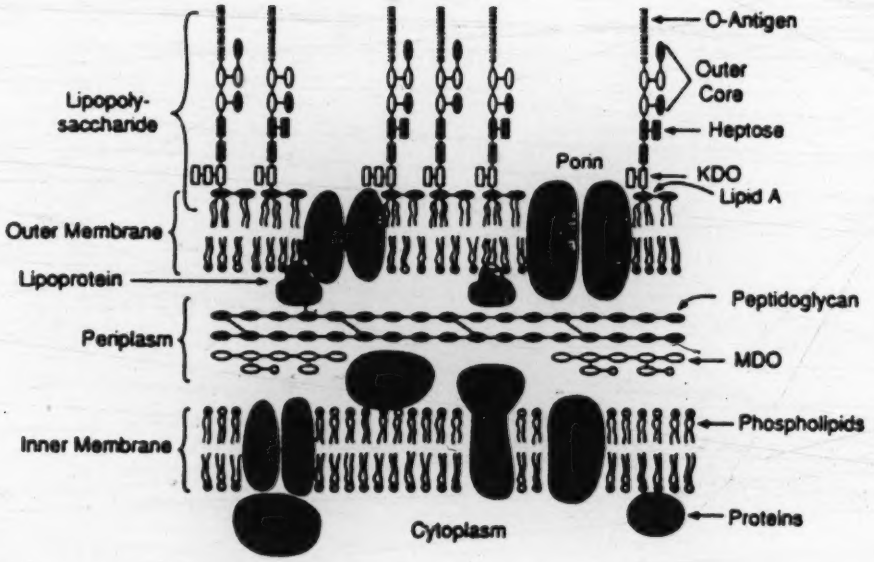


Fig. 1.8.: Representation of the envelope of Gram-negative bacterium.

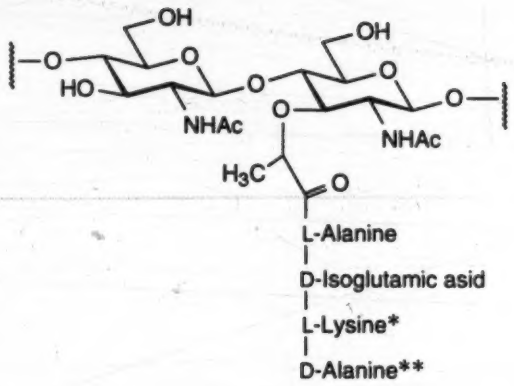


Fig. 1.7: Chemical structure of a peptidoglycan. Cross-links are formed by tetrapetide chains between the amino group of the lysine (*) on one chain and the C-terminal carboxyl group of the another (**) on another tetrapeptide.

Fig. 1.8.: Representation of the envelope of Gram-negative bacterium.

1.2.2. Mechanism of Bacterial Binding to the Host Cell Surface.

Bacteria adhering to host cells can cause infection. The resulting disease is the manifestation of the symptoms produced by the infection. In order to accomplish this, bacteria must reach a host surface and must adhere to host cells and colonize them. There

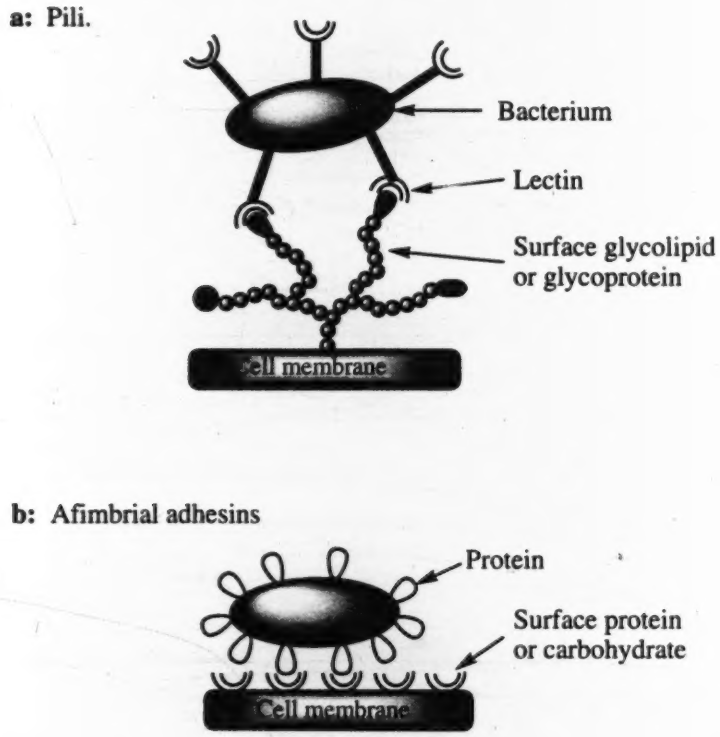


Fig. 1.9: Models for two types of bacterial adherence mechanisms.

are two common strategies that bacteria use to attach themselves to host cells as shown in Fig. 1.9 [16]. In one strategy the bacteria employ pili or fimbriae, rod-shaped protein structures that extend from the bacterial surface, to bind to host cell surface molecules, often carbohydrates. These pili allow the bacteria to make an initial loose contact with a host cell surface which then allows other bacterial surface proteins to bind more tightly.

The other mechanism that bacteria use involves so-called afimbrial adhesins, which are not rod-like structures, to bind tightly to host cells.

The glycoconjugates on mammalian cell membranes are the main target of bacterial adhesins [17]. Although the interaction between the adhesins and carbohydrate epitopes on cell surfaces is of low intrinsic affinity, it can develop high avidity utilizing multipoint interactions. The search for new antimicrobial therapies and vaccines is stimulated by the recurrence of an increasing number of antibiotic resistant bacteria [18]. A detailed understanding of the mechanism of bacterial pathogenesis and the interaction of adhesins with glycoconjugates at the initial stage of infection is essential for the conceptualization of new anti-infective agents. Blocking bacterial attachment is one strategy for combating infections.

1.2.3. Blocking Bacterial Attachment.

Bacteria employ their adhesins to bind to carbohydrates on susceptible host cell surfaces in the initial stage of infection (a, Fig. 1.10). Blocking the bacterial adhesins would prevent the infection. This can in principle be accomplished in two different ways. One way is by providing oligosaccharide inhibitors of low molecular weight and high affinity. These oligosaccharides would block the bacterial adhesins (b, Fig. 1.10). Another way is by providing soluble lectin-like molecules that can mask the carbohydrates on the cell surface (c, Fig. 1.10) [10].

Understanding as much as possible about the interaction between adhesins and carbohydrate receptors is essential for the development of adhesion blocker. Unfortunately, methods for studying the interactions between oligosaccharides and proteins

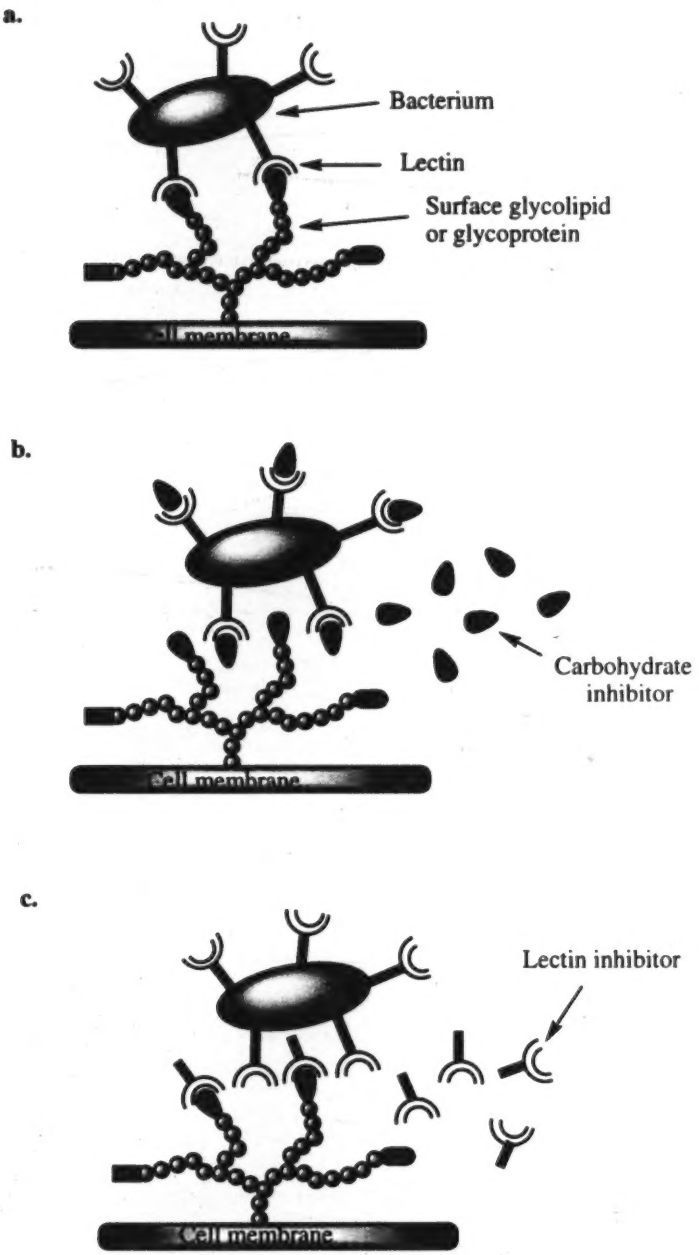


Fig. 1.10: Models for the strategies for blocking bacterial attachment.

are very limited. Crystallography and NMR spectroscopy have been successfully applied to some carbohydrate-protein complexes [19, 20] but they are complex and unlikely to be general methods for revealing all oligosaccharide-protein molecular interactions. Chemical mapping has therefore been developed to study the features of carbohydrate epitopes that are essential for recognition [21].

1.3. *Pseudomonas aeruginosa.*

1.3.1. *Pseudomonas aeruginosa* Bacteria.

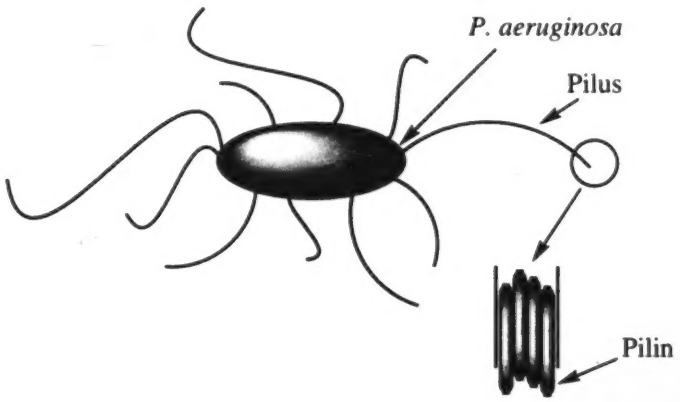


Fig. 1.11: Model for the structure of *P. aeruginosa.*

Pseudomonas aeruginosa is a Gram-negative rod shaped bacterium commonly found in patients with cystic fibrosis. There are many (from one or two to several hundred) thin nonflagellar protein filaments, called pili, on its surface (Fig. 1.11). *P. aeruginosa* causes infection resulting in opportunistic respiratory disease in cancer, cystic fibrosis and intensive care patients [22]. *P. aeruginosa*, like many other microorganisms, has several gene-products that may function as adhesins [23]. The pilus was one of the

first *P. aeruginosa* gene-products to be associated with pathogenicity because of its ability to allow bacterial adherence to human epithelial cell surfaces [24]. There are at least five distinct pili serotypes that exist among naturally occurring strains of *P. aeruginosa.* The amino acid sequences of four of the five different pili are now known [25]. The four known pili are from strain *P. aeruginosa* K (PAK), strain *P. aeruginosa* O (PAO), CD4 and PA103. PAO, CD4 and PA103 are serologically identical (PAO type) whereas PAK is a unique serotype [26].

1.3.2. *P. aeruginosa* PAK Pili.

Bacterial nonflagellar filamentous appendages have been referred to as threads, filaments, bristles, cilia, fibrillae, fuzz, colonization factor antigens, adhesins, fimbriae and pili since their discovery by both Anderson [27] and Houwink [28] in 1949. The designation "pili" (Latin for hair-like structure) was introduced by Brinton in 1959 [29]. There are several types of pili with no general agreement on any specific classification scheme for them. PAK pili are an example of type-IV pili which are multifunctional virulence factors in many bacterial pathogens [12]. *P. aeruginosa* polar pili are flexible filaments of 5.2 nm diameter and 2.5 μm average length and consist of many subunits, called pilins (15 kD), arranged in a helix of five subunits per turn [30] (Fig. 1.12). Each pilin is 144 amino acid residues in length [31] (Fig. 1.13). Only the pilins on the tip of the pili can bind to carbohydrates. The binding domain peptide of the pilin is located at the C-terminus and consists of a 17 amino acid sequence containing a disulfide bridge [24 b].

1.3.3. Receptors for *P. aeruginosa* PAK Pili.

P. aeruginosa employs several adhesins to mediate attachment to the cell surface. Studies on binding, attachment and colonization properties indicate that the pilus is the

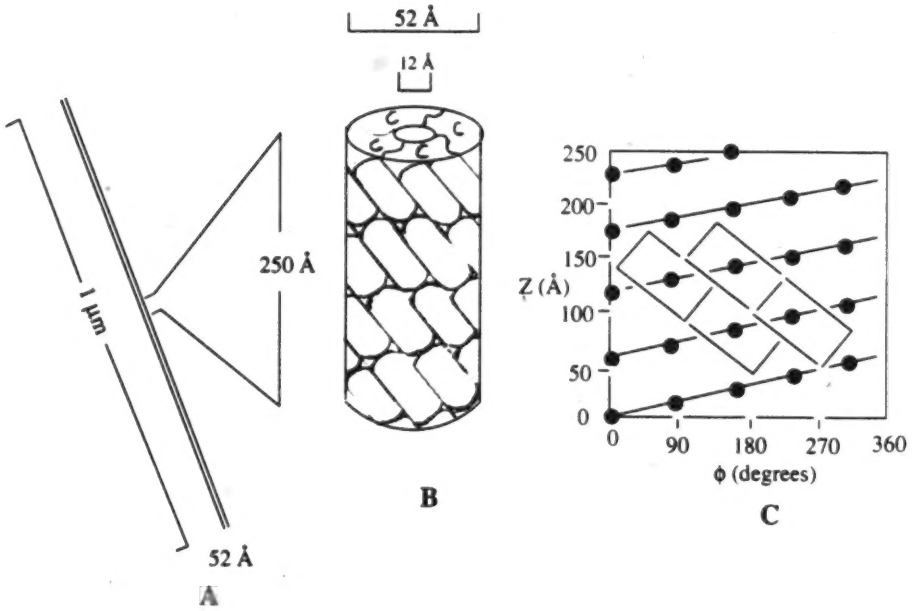


Fig. 1.12: Model for pilus structure based on X-ray diffraction and hydrodynamic data. A) A scaled representation of the intact pilus; B) A schematic representation showing one of several possible subunit orientations and shapes; C) Surface lattice representation indicating the 5 unit per turn symmetry indicated by X-ray diffraction data. One dimer is outlined in heavy lines. z represents the distance along an axis parallel to the long axis of the pilus; ϕ is the rotation.

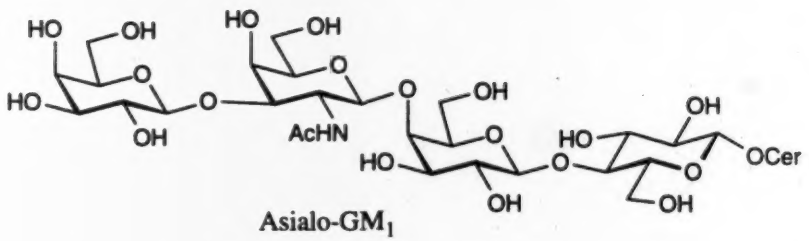
dominant adhesin responsible for initiating infection as mentioned above [32]. In particular, the pilus adhesin is a significant virulence factor in animal infection models [33]. The mechanism of the pilus adherence to the epithelial cell surface has so far not been fully characterized.

In vitro studies conducted using a thin-layer chromatogram-bacterial overlay assay have demonstrated that *P. aeruginosa* binds to the glycosphingolipids asialo-GM_1 and asialo-GM_2 [34] (Fig. 1.14) as well as to some non-carbohydrate receptors such as

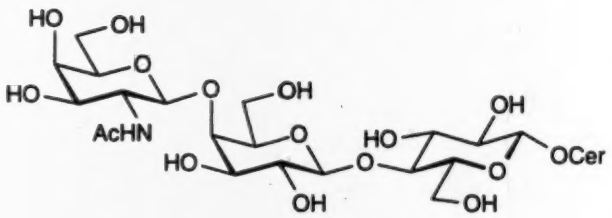
N-Mephe-Thr-Leu-Ile-Glu-Leu-Met-Ile-Val-Val-Ala-Ile-Ile-Gly-Ile-Leu-Ala-
Ala-Ile-Ala-Ile-Pro-Gln-Tyr-Gln-Asn-Tyr-Val-Ala-Arg-Ser-Glu-Gly-Ala-Ser-
Ala-Leu-Ala-Ser-Val-Asn-Pro-Leu-Lys-Thr-Thr-Val-Glu-Glu-Ala-Leu-Ser-
Arg-Gly-Trp-Ser-Val-Lys-Ser-Gly-Thr-Gly-Thr-Glu-Asp-Ala-Thr-Lys-Lys-
Glu-Val-Pro-Leu--Gly-Val-Ala-Al-Asp-Ala-Asn-Lys-Leu-Gly-Thr-Ser-Ile-Ala-
Leu-Lys-Pro-Asp-Pro-Ala-Asp-Gly-Asp-(Thr,Ala)-Ile-Thr-Leu-Thr-Phe-Thr-
Met-Gly-Gly-Ala-Gly-Pro-Gly-Lys-Asn-Lys-Lys-Ile-Ile-Thr-Leu-Thr-Arg-Thr-
Ala-Ala-Asp-Gly-Leu-Trp-Lys-Cys-Thr-Ser-Asp-Gln-Asp-Glu-Gln-Phe-Ile-
Pro-Lys-Gly-Cys-Ser-Lys-COOH

Fig. 1.13: Primary structure of *P. aeruginosa* pilin.

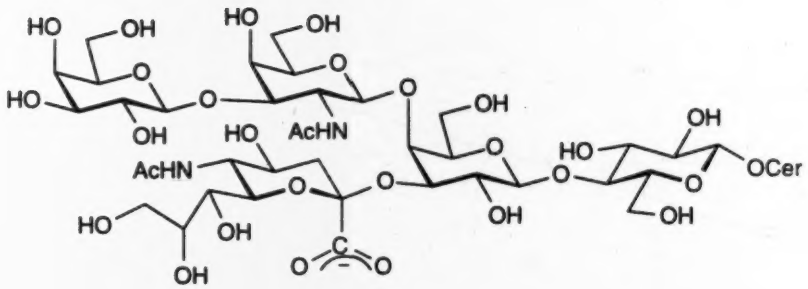
anti-*P. aeruginosa* pilus monoclonal antibodies (MABs) PK99H and Fm16 [35]. More recently, *P. aeruginosa* binding to asialo-GM_1 and asialo-GM_2 has been confirmed by several studies [36]. The minimum ligand structure in asialo-GM_1 and asialo-GM_2 is β-D-GalNAc-(1→4)-β-D-Gal carbohydrate sequence [34a, 35]. An interesting observation was the lack of binding to the gangliosides GM_1, GM_2, GD_{1a}, GD_{1b}, and Gt_{1d}, even though they have the β-D-GalNAc-(1→4)-β-D-Gal sequence (Fig. 1.15). It was proposed that the sialyl residue of these gangliosides interferes with the recognition process when BSA was used as a blocking agent in the assays [17]. On the other hand, *P. aeruginosa* can also bind to sialic acid-containing glycosphingolipids and lactosylceramide when gelatin was used as a blocking agent [37]. One explanation was that the use of BSA as a blocking agent suppressed the binding specificities.



Asialo-GM_1



Asialo-GM_2



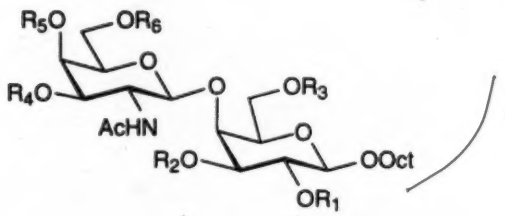
GM_1

Fig. 1.14: Structures of asialo-GM_1, asialo-GM_2 and GM_1.

The binding of *P. aeruginosa* pili to glycosphingolipids is a tip-associated event involving the C-terminal region of the structural pilin subunit [38]. Studies have found that *P. aeruginosa* binds to the synthetic β-D-GalNAc-(1→4)-β-D-Gal disaccharide [39] and even better to synthetically modified (mono-*O*-alkyl) β-D-GalNAc-(1→4)-β-D-Gal analogs [40] (Fig. 1.16). The IC_{50} of mono-*O*-propyl-β-D-GalNAc-(1→4)-β-D-Gal (IC_{50} = 8 μM)

Lactosyl ceramide	β-Gal(1-4)β-Glc(1-1)Cer
Asialo-GM_1	β-Gal(1-3)β-GalNAc(1-4)β-Gal(1-4)β-Glc(1-1)Cer
Asialo-GM_2	β-GalNAc(1-4)β-Gal(1-4)β-Glc(1-1)Cer
GM_1	β-Gal(1-3)β-GalNAc(1-4)[α-Neu5Ac(2-3)]β-Gal(1-4)β-Glc(1-1)Cer
GM_2	β-GalNAc(1-4)[α-Neu5Ac(2-3)]β-Gal(1-4)β-Glc(1-1)Cer
GD_{1a}	αNeu5Ac2-3βGal1-3βGalNAc1-4(αNeu5Ac2-3)βGal1-4βGlc1-1Cer
GD_{1b}	βGal1-3βGalNAc1-4(αNeu5Ac2-8αNeu5Ac2-3)βGal1-4βGlc1-1Cer
GT_{1b}	αNeu5Ac2-3βGal1-3βGalNAc1-4(αNeu5Ac2-8αNeu5Ac2-3)βGal1-4βGlc1-1Cer
Cad	βGalNAc1-4(αNeu5Ac2-3)βGal1-4βGlcNAc1-3-βGal1-4βGlc1-1Cer

Fig. 1.15: Structures of some glycolipids tested for binding to *P. aeruginosa*.



	R^1	R^2	R^3	R^4	R^5	R^6
1	**Propyl**	H	H	H	H	H
2	H	Propyl	H	H	H	H
3	H	H	Propyl	H	H	H
4	H	H	H	Me	H	H
5	H	H	H	H	Me	H
6	H	H	H	H	H	Me

Fig. 1.16: Structures of mono-*O*-alkyl-β-D-GalNAc-(1→4)-β-D-Gal analogs tested for binding to *P. aeruginosa*.

is up to 10 times lower than that of the native β-D-GalNAc-(1→4)-β-D-Gal disaccharide (IC_{50} = 79 μM).

The binding affinity between carbohydrates and proteins is generally weak, with association constants in the range of 10^3-10^4 M^{-1} [41]. The average carbohydrate-binding domain spans two or three sugar units with the sugars ususally only partially buried in the combining site [42]. Consequently, a significant proportion of the sugar molecule may be found at the interface of protein and solvent. Several investigations have shown that replacement of the key polar groups by hydrogen, halide, amino, or methoxy groups often leads to less active compounds [43]. However, it has been shown that hydrophobic interactions in the periphery (4-6 Å) of the carbohydrate-protein binding site can be used to increase binding affinities [44, 21 b].

1.4. Multivalency.

Protein-carbohydrate interactions facilitate fundamental cell-cell recognition events in processes as diverse as host-pathogen interactions, fertilization, development, and the mounting of an immune response [45]. Despite their importance in cell recognition, individual protein-carbohydrate interactions are generally weak as mentioned above [41]. Both affinity and specificity are critical components of cell-cell recognition in biological systems. The low affinity and often relaxed specificity of individual protein-carbohydrate interactions is difficult to reconcile with the striking diversity of oligosaccharide structures that are involved in specific recognition processes [4]. Nature often assembles multiple protein-saccharide complexes to provide the necessary avidity to enhance the strength of cell surface binding. There are several possible ways that multiple protein-carbohydrate interactions can occur as shown in Fig. 1.17 [46].

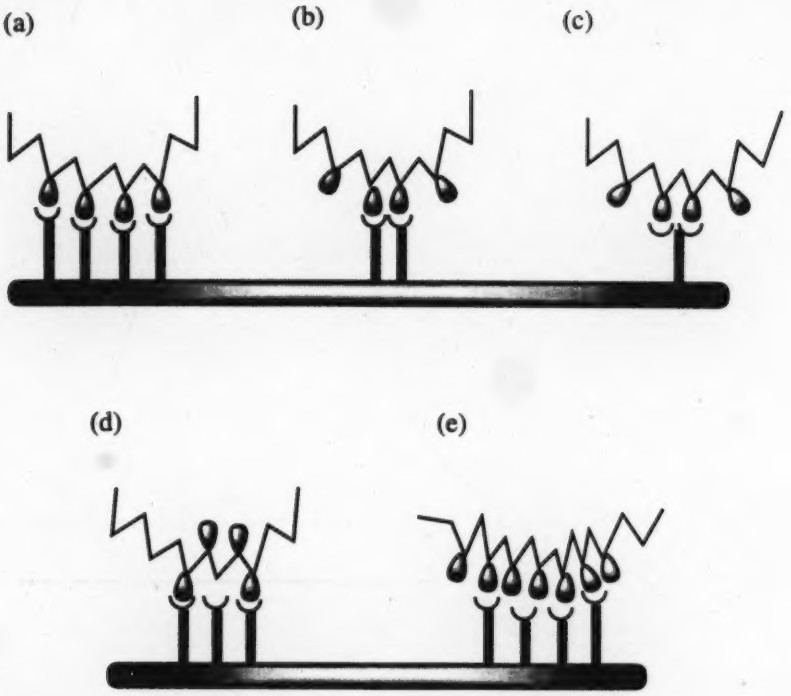


Fig. 1.17: Specific recognition in multivalent interactions. Cells can use several strategies to bind to a multivalent ligand: (a) forming a cluster of many monovalent receptors on a small area of the cell surface; (b) using oligomeric receptors; or (c) using receptors with more than one saccharide-binding site. Multivalent ligands with incompatible relative orientation (d) or spacing (e) of the saccharide units in the multivalent array will not bind tightly.

Multivalent carbohydrate displays are widespread in nature. They occur in the highly glycosylated mucins, the carbohydrate coats of bacteria, viruses and other pathogens as well as the outer membranes of mammalian cells. The advantages of a multivalent binding system confered in a biological setting are as follows. First, since the characteristics of binding can be tuned by alteration of the individual saccharide residues or their relative orientations, recognition events can be readily and flexibly modulated. Second, the kinetics of multipoint attachment will be different from those involved in the formation of a single receptor-ligand binding event. Third, multivalent interactions are expected to be more resistant to shear stress, a feature that may be significant for some cell-

cell recognition processes. And finally, after the first binding site on the cell surface docks the first sugar unit, the second one is present in a higher effective local concentration. The entropy change for binding of the second sugar unit to the second binding site is much less unfavorable than the intrinsic binding entropy change. The second binding will be thermodynamically much more favorable than the first one in terms of its binding free energy [47].

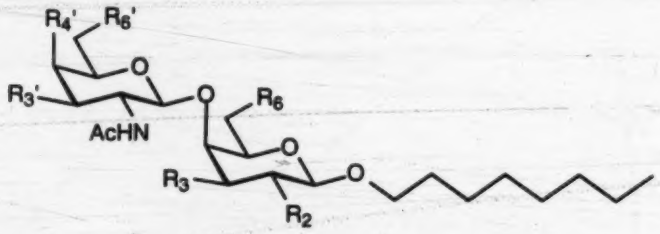
1.5. Objective.

Many bacteria adhere to mammalian cells through the recognition of cell-surface carbohydrates by bacterial pili proteins, thus initiating an infection. The corollary is that small molecules that resemble the oligosaccharide structures recognized by the bacterial pili can be inhibitors of such adhesion. Specifically, the binding of the *P. aeruginosa* strains PAK and POK pili to the central disaccharide β-D-GalNAc-(1→4)-β-D-Gal of the natural target glycolipid pentasaccharide asialo-GM_1 should be inhibitable. The main objective of this part of the thesis was to develop inhibitors of adhesion that are simpler and have higher affinity than the natural oligosaccharide ligands. Two synthetic approaches were used.

1.5.1. Design and Synthesis of Mono-deoxygenated Octyl β-D-GalNAc-(1→4)-β-D-Gal Analogs as Potential Inhibitors of Bacterial Adhesion.

In order to gain a more detailed understanding on a molecular level of the pilus-carbohydrate interaction, as well as to contribute to the understanding of carbohydrate-protein interaction in general, a chemical mapping approach employing single hydroxy-modified octyl β-D-GalNAc-(1→4)-β-D-Gal analogs was chosen. That is, one at a time, each of the hydroxy groups of octyl β-D-GalNAc-(1→4)-β-D-Gal disaccharide was replaced by hydrogen, methoxy or propoxy group. In this thesis, six monodeoxy derivatives of

octyl β-D-GalNAc-(1→4)-β-D-Gal were synthesized as potential competitive inhibitors of *P. aeruginosa* binding to asialo-GM_1 (Fig. 1.18).



	R_2	R_3	R_6	R_3'	R_4'	R_6'
1	**H**	OH	OH	OH	OH	OH
2	OH	**H**	OH	OH	OH	OH
3	OH	OH	**H**	OH	OH	OH
4	OH	OH	OH	**H**	OH	OH
5	OH	OH	OH	OH	**H**	OH
6	OH	OH	OH	OH	OH	**H**

Fig. 1.18: Six mono-deoxygened octyl β-D-GalNAc-(1→4)-β-D-Gal analogs

1.5.2. Synthesis of Simple Multivalent β-D-GalNAc-(1→4)-β-D-Gal Oligomers as Probes for Investigating the Interaction of *P. aeruginosa* Pili with Multivalent Receptors.

To obtain a better insight into the nature of the interaction of *P. aeruginosa* PAK pili with multivalent carbohydrate β-D-GalNAc-(1→4)-β-D-Gal ligand and more information for the design of more effective anti-adhesive therapeutics for the prevention of infection, five multivalent β-D-GalNAc-(1→4)-β-D-Gal analogs as templates for investigating the interactions of *P. aeruginosa* PAK pili with multivalent acceptors (Fig. 1.19) were synthesized.

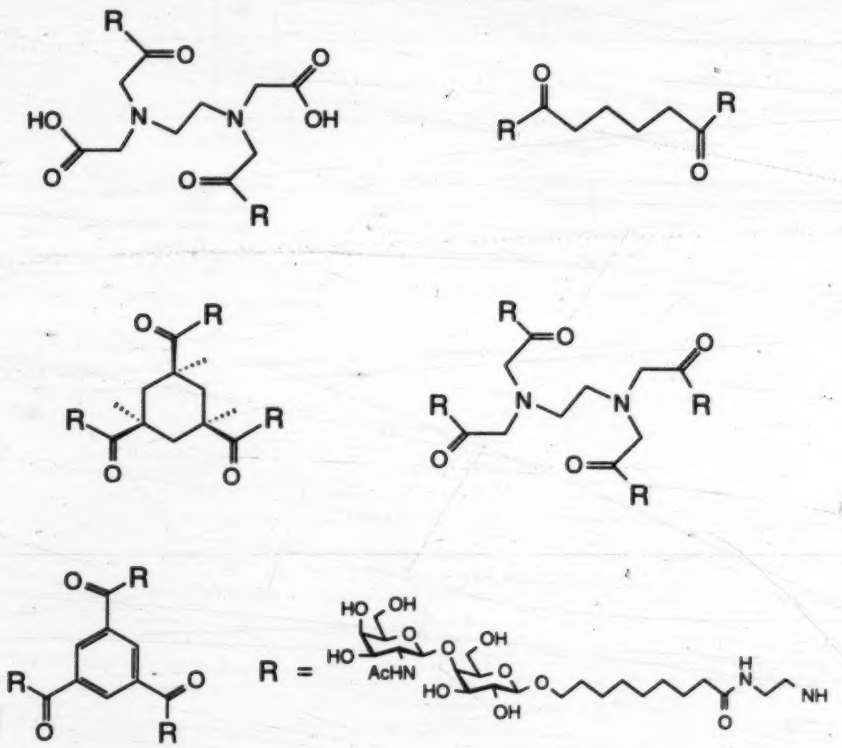


Fig. 1.19: Five multivalent β-D-GalNAc-(1→4)-β-D-Gal analogs.

1.6. Potential Significance of Results.

Two series of analogs of β-D-GalNAc-(1→4)-β-D-Gal sequence were chemically synthesized (in Chapters 2 and 3) for investigating the interaction of *P. aeruginosa* PAK pili with mono- and multivalent receptors. The first series consisted of six mono-deoxygenated octyl β-D-GalNAc-(1→4)-β-D-Gal analogs **57**, **60**, **63**, **67**, **70** and **74** and the second series included five multivalent β-D-GalNAc-(1→4)-β-D-Gal oligomers **85-89**.

The evaluations of the biological activity of these compounds will be performed in collaboration with Professor Randall T. Irvin (Department of Medical Microbiology and Infectious Diseases, University of Alberta). These evaluations await the development of new and sensitive biosensor-based assay in a research project funded by PENCE.

The results obtained from the binding studies with the mono-deoxygenated octyl β-D-GalNAc-(1→4)-β-D-Gal analogs will help us identify the key polar groups responsible for pili binding. The hydroxy functions that appear not to be participating in the binding will be replaced by suitable hydrophobic groups. The 2-deoxy analog **74** is expected to be a strong inhibitor since the lipophilic region, resulting from the 2-deoxygenation and octyl aglycon, is known to result in higher binding affinity. Finally, the multivalent β-D-GalNAc-(1→4)-β-D-Gal analogs will be evaluated to determine the effect of the multivalency on the binding affinity. We expect these multivalent compounds to bind strongly with pili, compared with their monovalent counterparts, since a pilus contains a number of pilins at the binding tip.

Chapter 2

Design and Synthesis of Mono-deoxygenated Octyl β-D-GalNAc-(1→4)-β-D-Gal Analogs as Potential Inhibitors of Bacterial Adhesion

2.1. Introduction

2.1.1. Synthetic strategy for the synthesis of mono-deoxy octyl β-D-GalNAc-(1→4)-β-D-Gal analogs

This chapter describes the synthesis of the mono-deoxy octyl β-D-GalNAc-(1→4)-β-D-Gal analogs **57**, **60**, **63**, **67**, **70** and **74** (Fig. 2.1) as potential inhibitors of bacterial adhesion. The synthetic strategy (Scheme 2.1) included: 1) the synthesis of the mono-deoxy galactosamine donors **14**, **25**, **37** and the mono-deoxygenated octyl galactopyranoside acceptors **46**, **49**, **53**; 2) β-glycosylation of octyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (**54**) with the three mono-deoxy galactosamine donors and β-glycosylation of the three mono-deoxygenated octyl galactopyranoside acceptors with methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside as the donor (**64**); and 3) the removal of protecting groups on the disaccharides **55**, **58**, **61**, **65**, **68** and **71**.

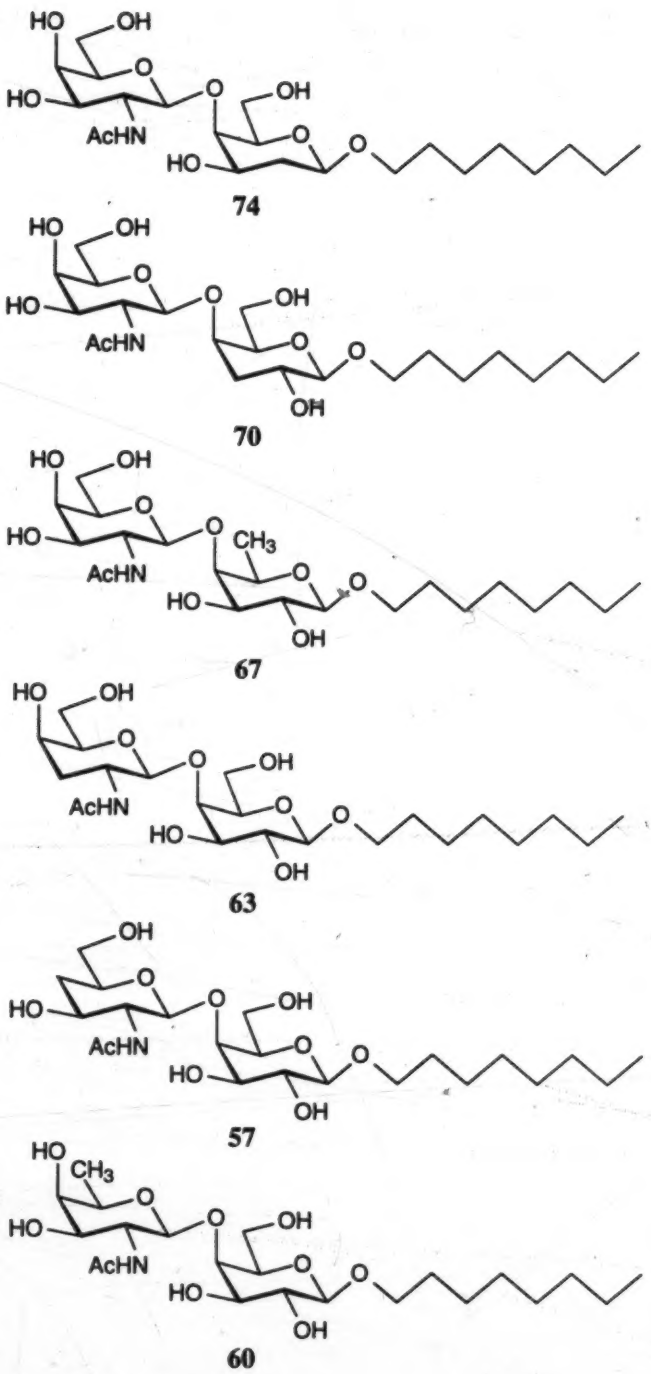
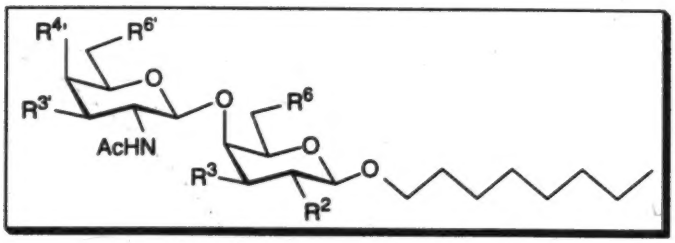


Fig. 2.1: Structures of six mono-deoxygenated octyl β-D-GalNAc-(1→4)-β-D-Gal target compounds.

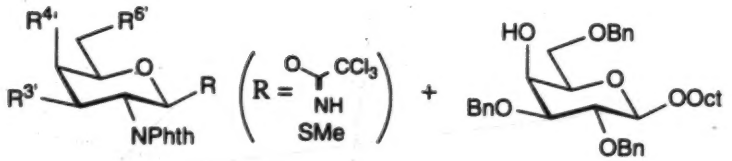
Barton deoxygenation reactions were key in this synthetic strategy. Thus, sugar alcohols were transformed into thiocarbonyl derivatives which were then reduced using tributyltin hydride and AIBN as the initiator to afford the required deoxy-compounds (Scheme 2.1b) [49].



Mono-deoxy disaccharides
74, 70, 67, 63, 57 and 60

Deprotection

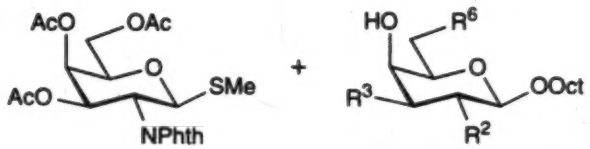
Glycosylation



Mono-deoxy glycosyl donors
14, 25, 37, 38

54

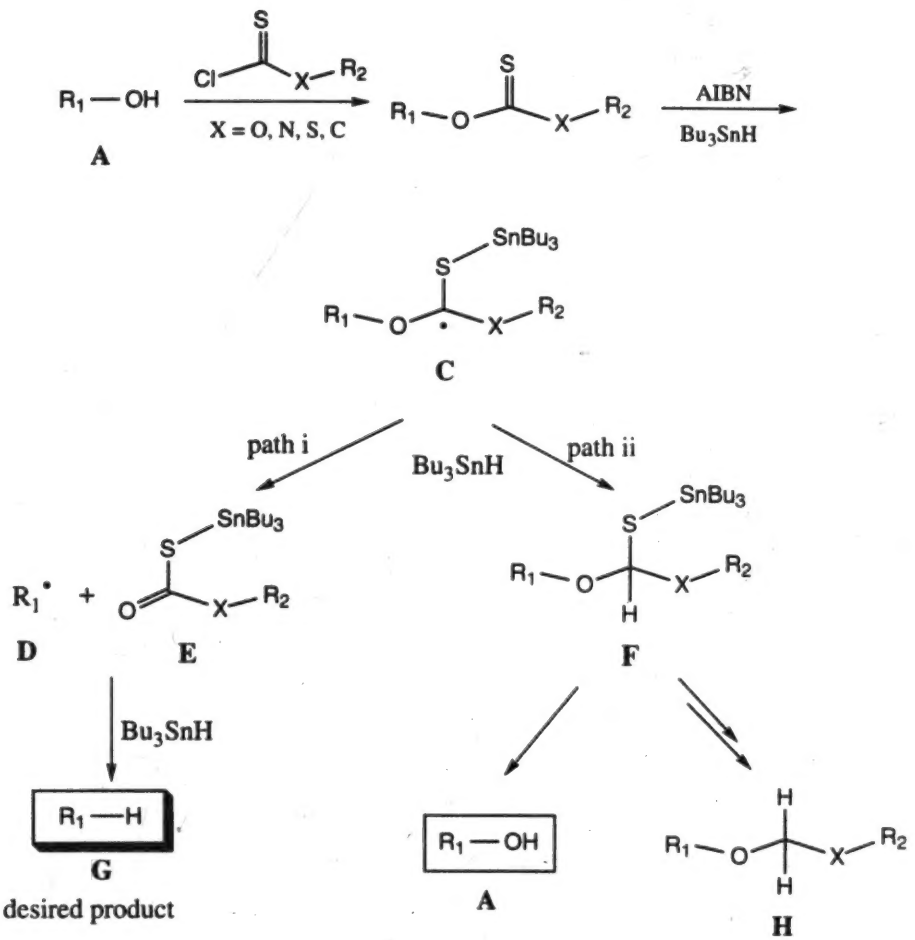
or



64

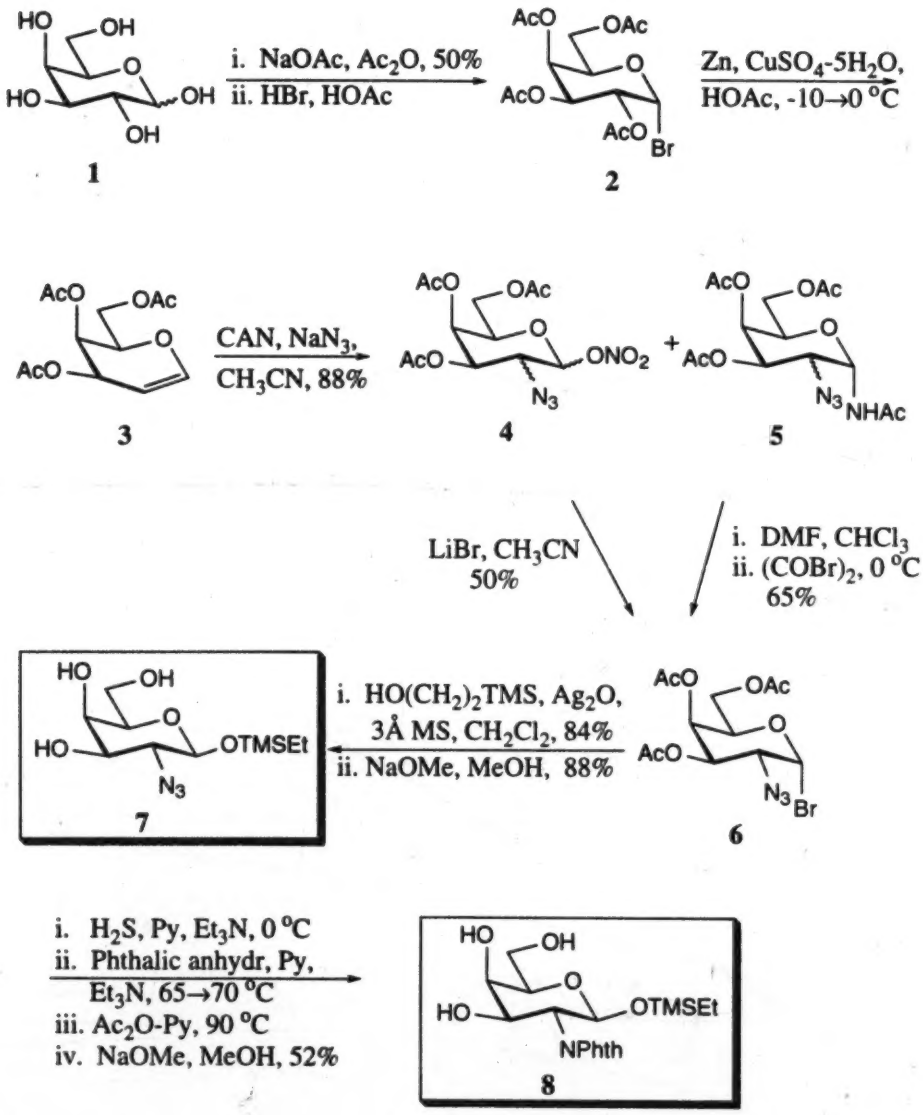
Mono-deoxy glycosyl acceptors
46, 49, 53

Scheme 2.1a: Synthetic strategy for the preparation of mono-deoxygenated octyl β-D-GalNAc-(1→4)-β-D-Gal analogs.



Scheme 2.1b: Barton radical deoxygenation reaction. It is a radical competive reaction. The reaction follows path i if radical **D** is more stable than **C**. Otherwise, the reaction proceeds via path ii.

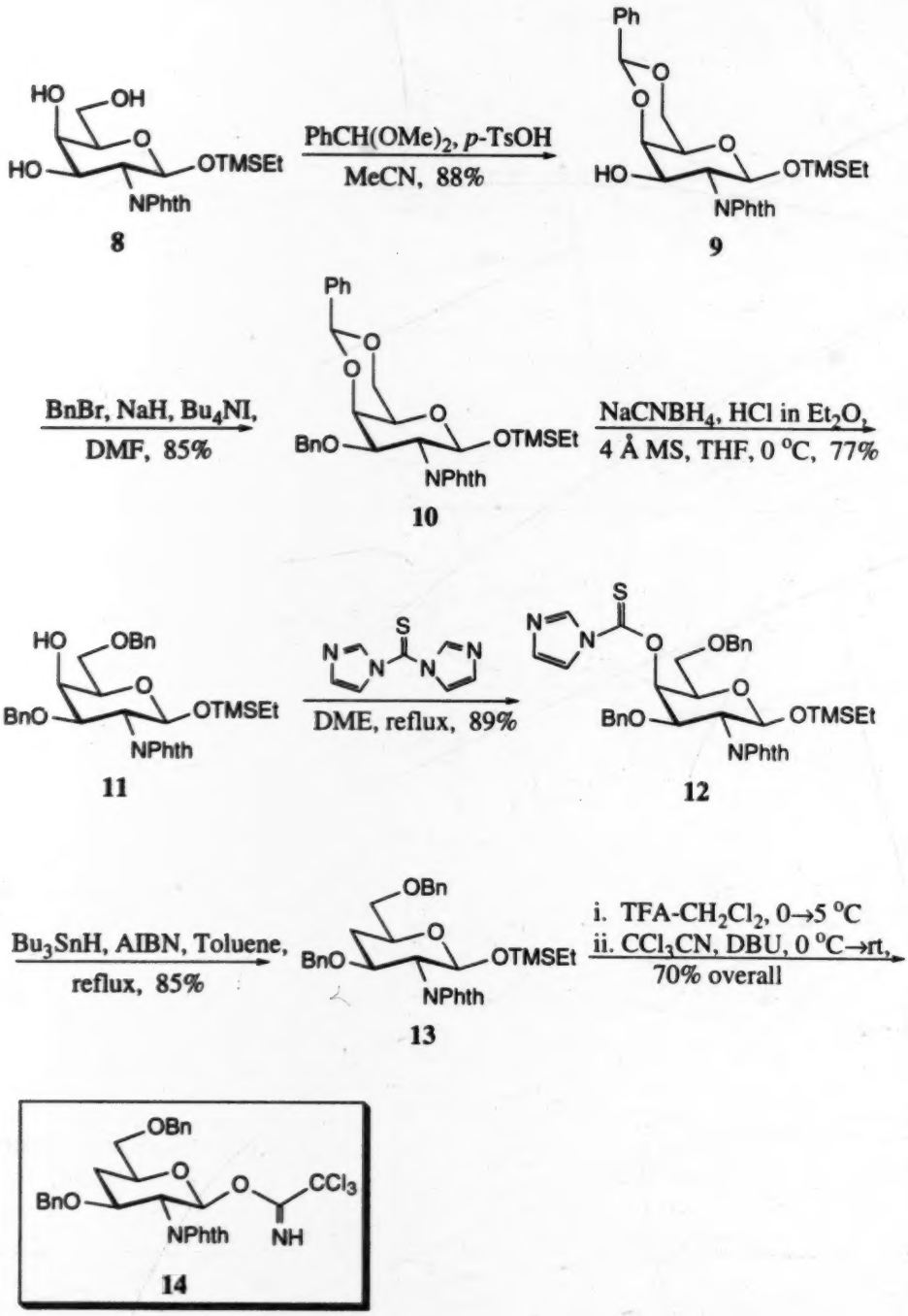
2.1.2. Preparation of Protected Mono-deoxy Galactosamine Glycosyl Donors.



Scheme 2.2: Preparation of compounds **7** and **8**.

The three protected mono-deoxy galactosamine glycosyl donors **14**, **25** and **37** were synthesized either from 2-(trimethylsilyl)ethyl 2-azido-2-deoxy-β-D-galactopyranoside (**7**) or 2-(trimethylsilyl)ethyl 2-deoxy-2-phthalimido-β-D-galactopyranoside (**8**). The 2-(trimethylsilyl)ethyl group (TMSEt) was chosen for the protection of the anomeric hydroxyl group because of its versatility. It is stable towards most reaction conditions employed in contemporary carbohydrate chemistry [50, 51] with the exception of strong Lewis acids. Conversion of a TMSEt glycoside into the corresponding hemiacetal [50], glycosyl chloride [52] or 1-*O*-β-acetate [50] proceeds in near quantitative yield. Further conversions of the hemiacetal into a glycosyl trichloroacetimidate [53] or fluoride [54] and of the 1-*O*-β-acetate into a thioglycoside [55] process in over 90% yields providing access to the most efficient glycosyl donors known today.

Compounds **7** and **8** were prepared as shown in Scheme 2.2. *per*-Acetylation of D-galactose (**1**) with hot NaOAc and Ac_2O followed by bromination using HBr (45% v/v in HOAc) 5 °C → rt gave tetra-*O*-acetyl-α-D-galactopyranosyl bromide **2** [56]. Reduction of **2** using zinc dust and HOAc, in the presence of NaOAc and $CuSO_4$-$5H_2O$ 0 °C → rt for 3 h provided the galactal **3** [57]. Azidonitration of 3,4,6-tri-*O*-acetyl-D-galactal (**3**) using CAN (ceric (IV) ammonium nitrate) and NaN_3 in MeCN at -15 °C for 20 h gave a mixture of **4** and **5** in 88% yield [58]. Treatment of **4** with a suspension of LiBr in MeCN at rt for 4 h yielded the bromide donor **6** (50%) [58]. The 1-acetamido compound **5** was rapidly converted to **6** by treatment with Vilsmeier reagent (*N,N*-dimethylbromoforminium bromide [59]) in an ice bath overnight [58a] in 65% yield. The coupling reaction of the bromide **6** with 2-(trimethylsilyl)ethanol in the presence of Ag_2O as a promoter in dry CH_2Cl_2 at rt overnight gave the corresponding TMSEt glycoside (84%) [50]. Deacetylation using NaOMe in MeOH yielded **7** (88%). The reduction of the azide **7** to the amine using H_2S in the presence of Et_3N in pyridine at 0 °C for 8 h was essentially quantitative [58b, 60]. The product amine was used directly for the preparation of the

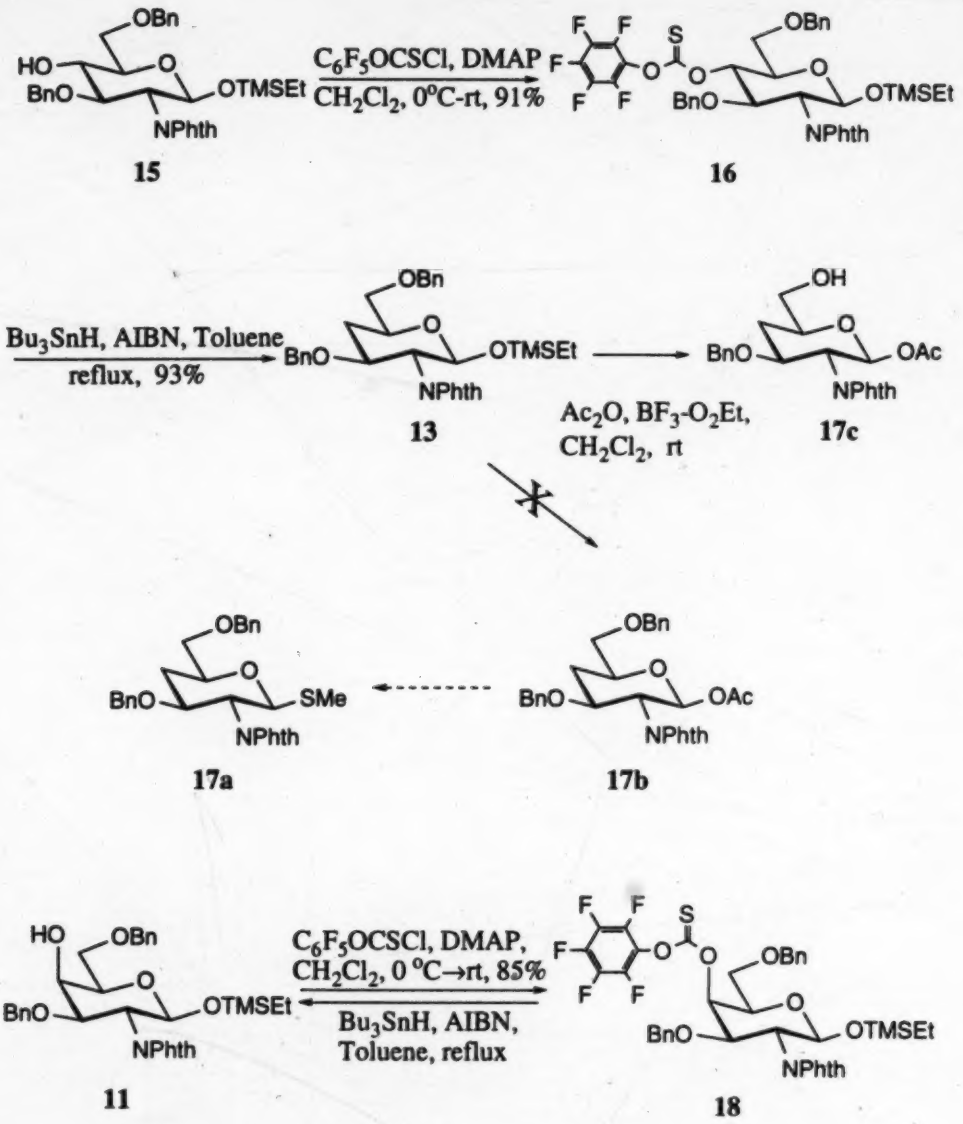


Scheme 2.3: Synthesis of the 4-deoxy trichloroacetimidate glycosyl donor **14**.

phthalimido compound **8**. Treatment of the amine with phthalic anhydride in the presence of Et_3N in pyridine at 70 °C for 2 h, followed by acetylation with Ac_2O and pyridine at 90 °C for 3 h gave the phthalimido product **8** (52% overall) [58b, 61].

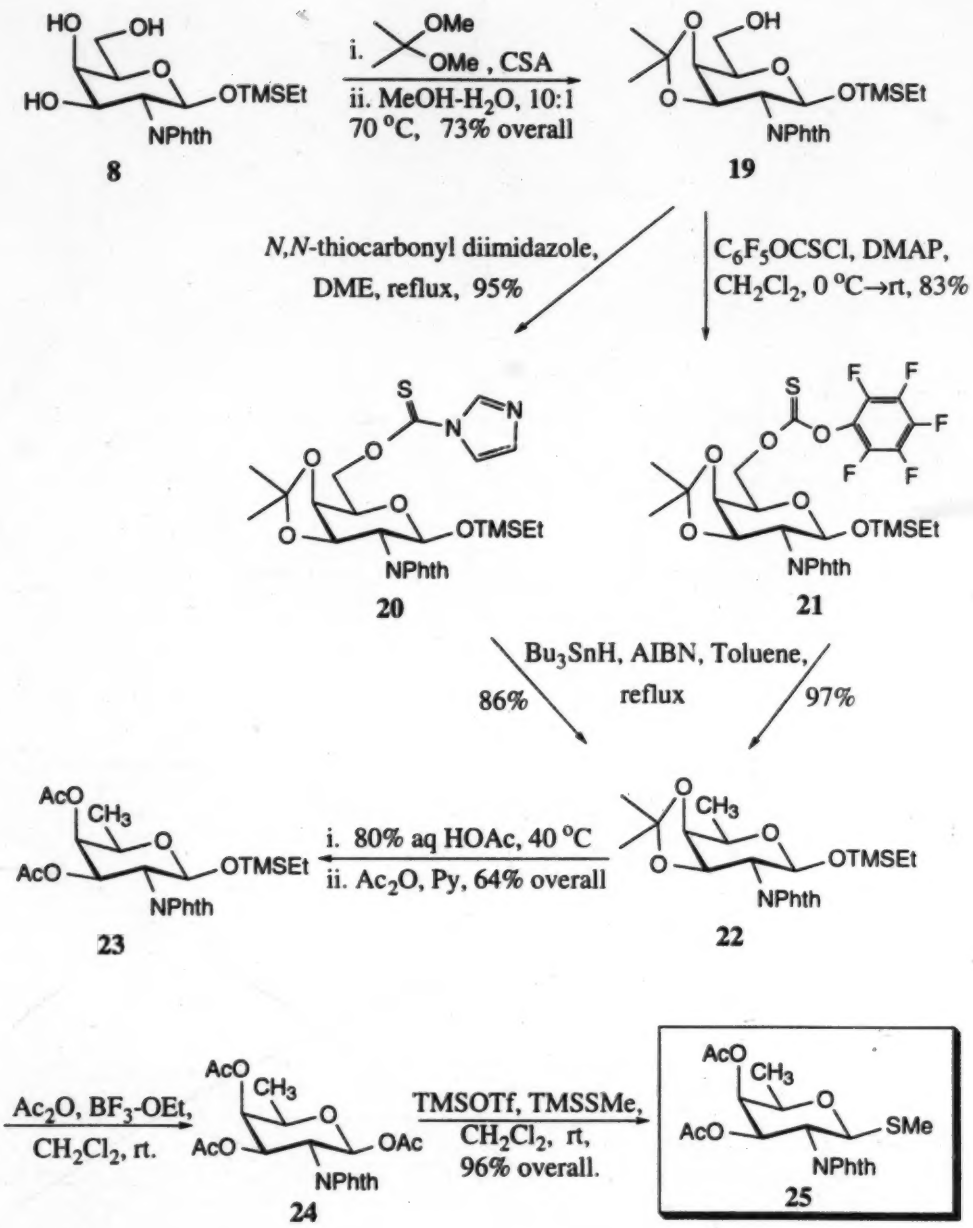
The synthesis of the 4-deoxygenated galactosamine donor **14** is shown in Scheme 2.3. Benzylidenation of **8** [61] using α,α–dimethoxytoluene and *p*-TsOH in MeCN at rt overnight, followed by column chromatography of the crude product, gave pure **9** (88%). Benzylation of **9** with BnBr and NaH in the presence of Bu_4NI [62] in DMF 0 °C → r.t. overnight gave **10** in 85% yield. Reductive ring opening of the benzylidene acetal in **10** using $NaCNBH_3$-HCl in the presence of 4 Å molecular sieves and methyl orange indicator in THF at 0 °C gave **11** in 77% yield [63]. The thiocarbonylation of **11** under basic conditions (NaH-CS_2-MeI) [64b] was not possible due to the instability of the phthalimido group. Instead, thiocarbonylation of **11** under mild conditions, using thiocarbonyldiimidazole in boiling DME overnight, gave **12** in 89% yield [64a]. Compound **12** was deoxygenated overnight with Bu_3SnH and 2,2'-azobisisobutyronitrile (AIBN) [65] in refluxing toluene under Ar and yielded the corresponding 4-deoxygenated galactosamine derivative **13** (85%). Conversion of **13** into a suitable glycosyl donor was achieved by hydrolysis of the TMSEt aglycon with TFA and CH_2Cl_2 (1:1) at 0 → 5 °C for 10 min [50] followed by concentration and co-evaporation with a mixture of toluene and PrOAc (1:1). Reaction with trichloroacetonitrile and 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) [66] 0 °C → rt then gave the trichloroacetimidate donor **14** in 70% overall yield [67].

In an alternative method for the synthesis of 2-(trimethylsilyl)ethyl 3,6-di-*O*-benzyl-2,4-dideoxy-2-phthalimido-β-D-galactopyranoside (**13**) the suitably protected 2-phthalimido-β-D-glucopyranoside **15** was used as the starting material (Scheme 2.4). Thiocarbonylation of **15** with pentafluorophenyl chlorothionoformate and DMAP in CH_2Cl_2 0 °C → rt for 5 h gave **16** in 91% yield. Radical reduction of **16** using Bu_3SnH



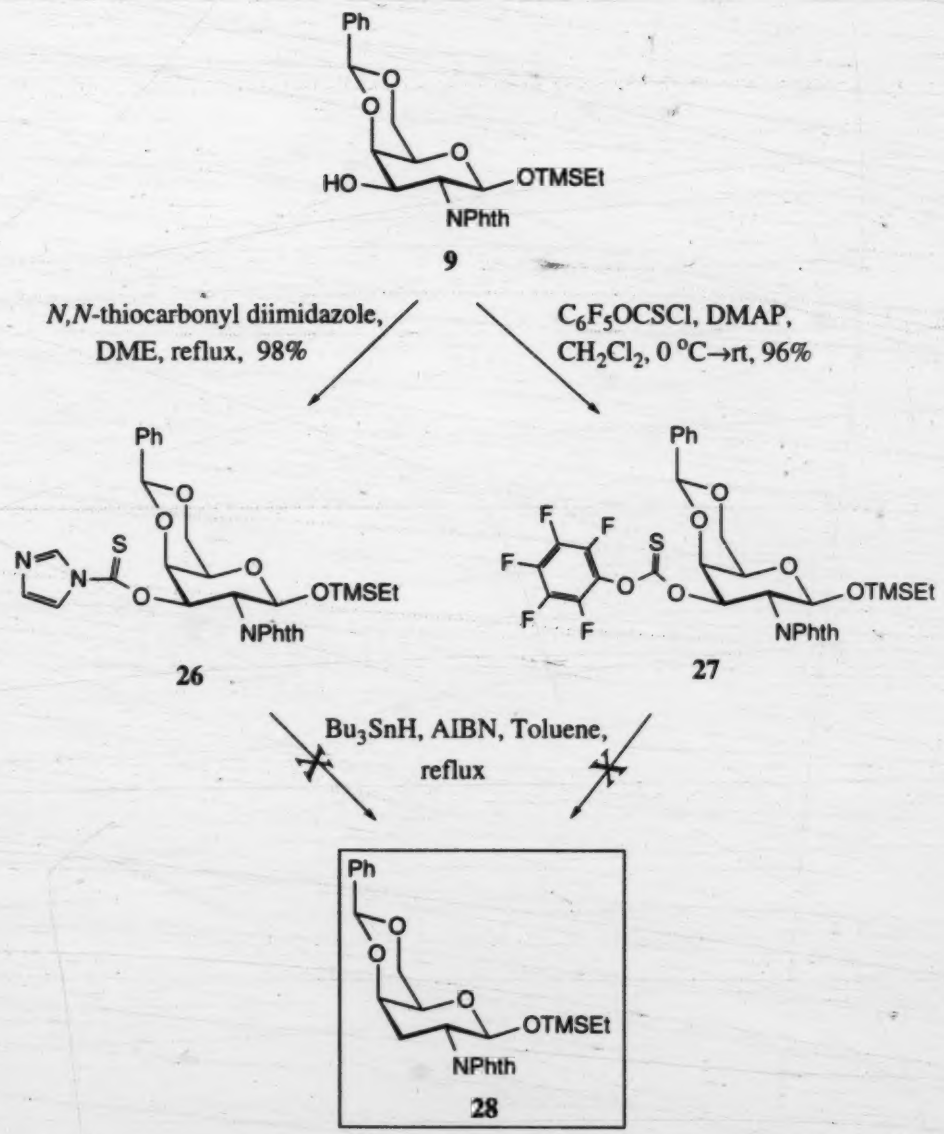
Scheme 2.4: Attempted synthesis of the 4-deoxy thioglycosyl donor **17a**.

and AIBN in refluxing toluene for 1 d yielded **13** (93%). The synthesis of the corresponding thioglycoside donor from **13** via the 1-*O*-β-acetate intermediate **17b** using Ac_2O and $BF_3.Et_2O$ in CH_2Cl_2 at rt failed and gave only **17c** [68]. On the other hand,



Scheme 2.5: Synthesis of the 6-deoxy thioglycosyl donor **25**.

thiocarbonylation of **11** with pentafluorophenyl chlorothionoformate and DMAP as above gave product **18** in 85% yield. Attempted radical reduction of **18** with Bu_3SnH and AIBN in refluxing toluene produced only the corresponding alcohol **11**. It was proposed that the



Scheme 2.6: Attempted synthesis of the 2,3-deoxy-2-phthalimido donor **28**.

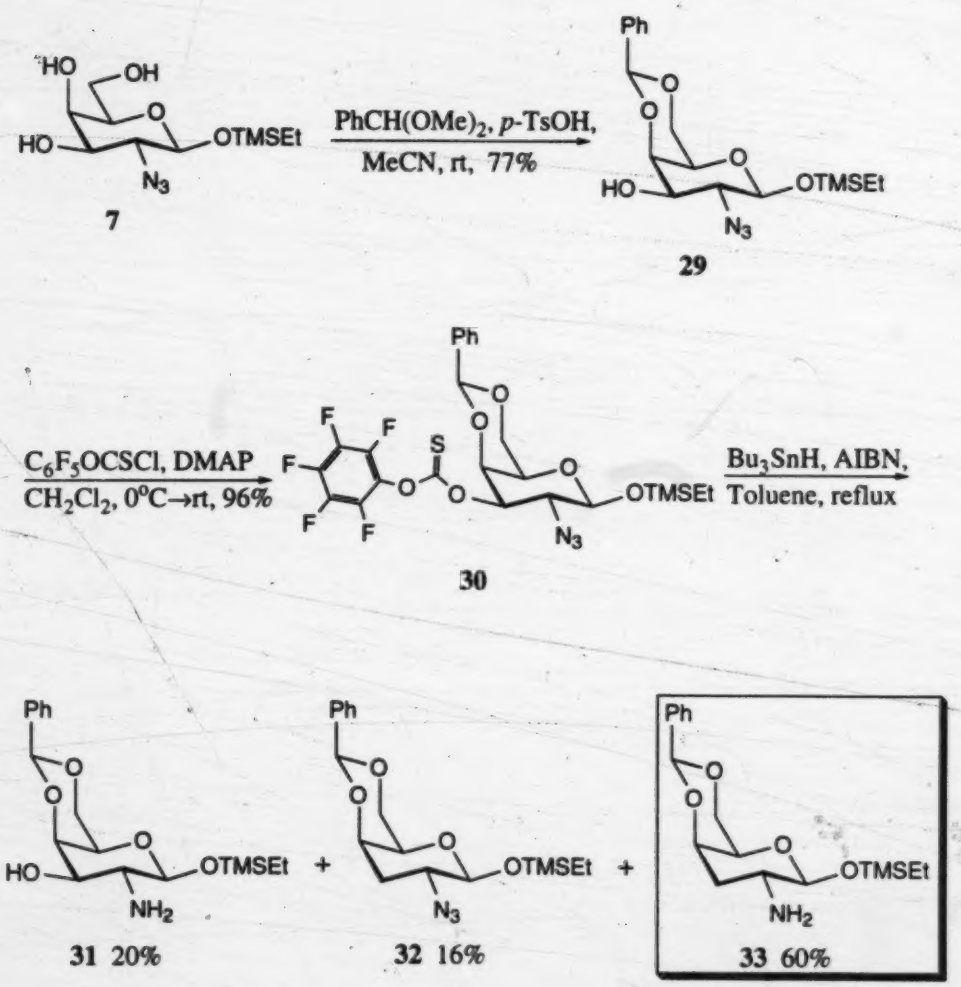
reaction followed path ii in scheme 2.1b.

The preparation of the methyl 2,6-dideoxy-2-phthalimido-1-thio-β-D-galactopyranoside donor **25** is shown in Scheme 2.5. Treatment of **8** with 2,2-dimethoxypropane and camphorsulphonic acid (*d*-CSA) [69] at rt 3 h and then with MeOH-H_2O (10:1) at 70 °C for 1 h gave **19** in 73% overall yield. Thiocarbonylation reactions of **19** with thiocarbonyldiimidazole in refluxing DME, or with pentafluorophenylchlorothionoformate and DMAP in CH_2Cl_2 at 0 °C → rt afforded **20** and **21** in 95% and 84% yields, respectively. Deoxygenations of **20** and **21** with Bu_3SnH and AIBN in refluxing dry toluene directly produced **22** (86% and 97%, respectively). De-isopropylidenation of **22** with 80% aq HOAc at 40 °C and then acetylation using Ac_2O and pyridine gave **23** (64%, overall yield from **22**). Compound **23** reacted with BF_3 etherate and Ac_2O in CH_2Cl_2 at rt to give crude **24** [50, 68]. Dry crude **24** was treated with TMSOTf and TMSSMe in CH_2Cl_2 at rt for 3 d to furnish the corresponding thioglycoside donor **25** (96% in two steps) [70].

The synthesis of the 2-(trimethylsilyl)ethyl 4,6-di-*O*-acetyl-2,3-dideoxy-2-phthalimido-β-D-xylopyranoside donor (**28**) from the 2-phthalimido galactopyranoside derivative **9** was not possible (Scheme 2.6). Thiocarbonyl compounds **26** and **27** can be easily prepared by the same procedure as described above in very high yields (98% and 96%, respectively). Attempted radical deoxygenations of **26** and **27** with Bu_3SnH and AIBN in refluxing toluene, however, did not produce **28**. This may be due to the phthalimido group at the C-2 position somehow preventing the C-3 radical formation during the radical deoxygenation reaction [49]. The reaction proceeded path ii in scheme 2.1b. The major product was starting material **9**.

The synthesis of the 3-deoxygenated galactosamine donor therefore had to begin with the azido galactopyranoside **7** [58a] (Scheme 2.7 and Scheme 2.8). Benzylidenation of **7** gave **29** in 77% yield (Scheme 2.7). Thiocarbonylation of **29** with

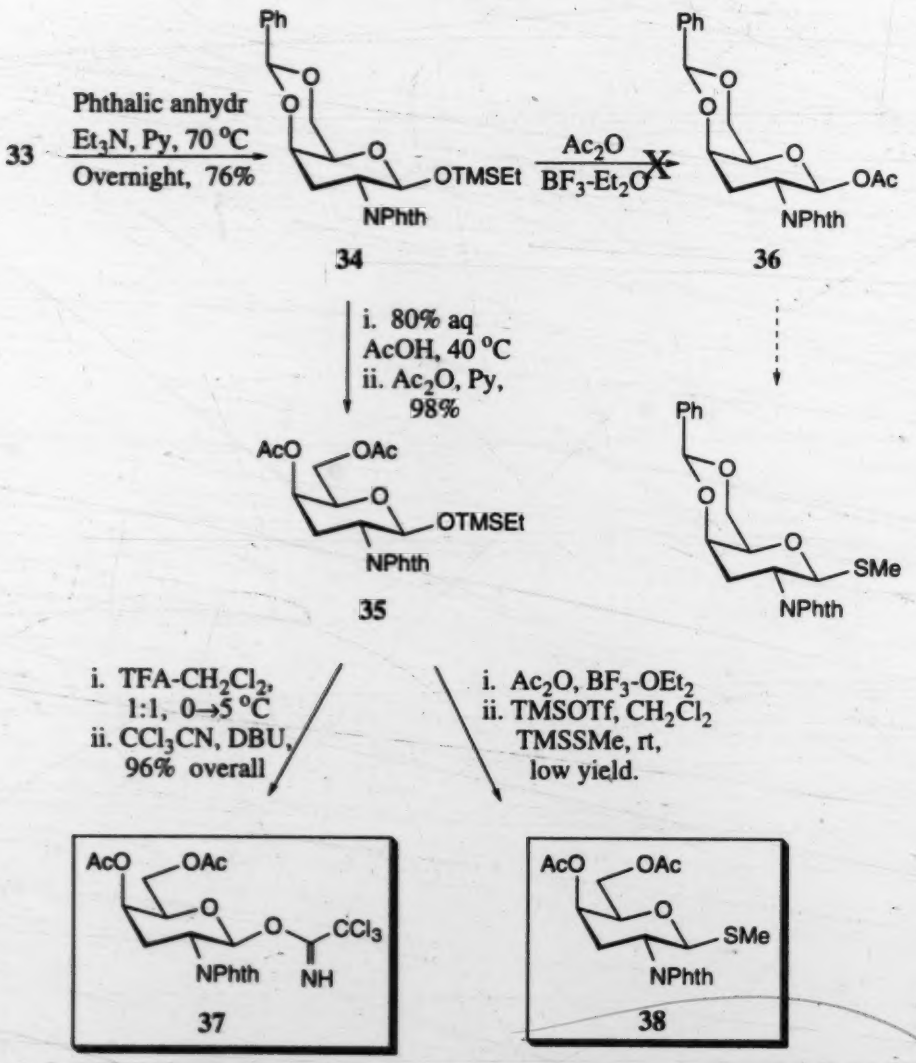
pentafluorophenylchlorothionoformate and DMAP gave **30** (96%) [71]. Treatment of **30** with Bu_3SnH and AIBN in dry toluene and refluxing for 1.5 d under Ar resulted in both



Scheme 2.7: Synthesis of the 2-amino-2,3-dideoxy-galactopyranoside **33**.

deoxygenation at C-3 and reduction of the azido group to furnish the deoxyamino product **33** (60%). The 3-hydroxy-2-amino product **31** (20%) and the 3-deoxy-2-azido product **32** (16%) were also isolated.

Compound **33** was treated with phthalic anhydride and Et_3N in pyridine at 65-75



Scheme 2.8: Synthesis of the 2,3-dideoxy-2-phthalamido donors **37** and **38**.

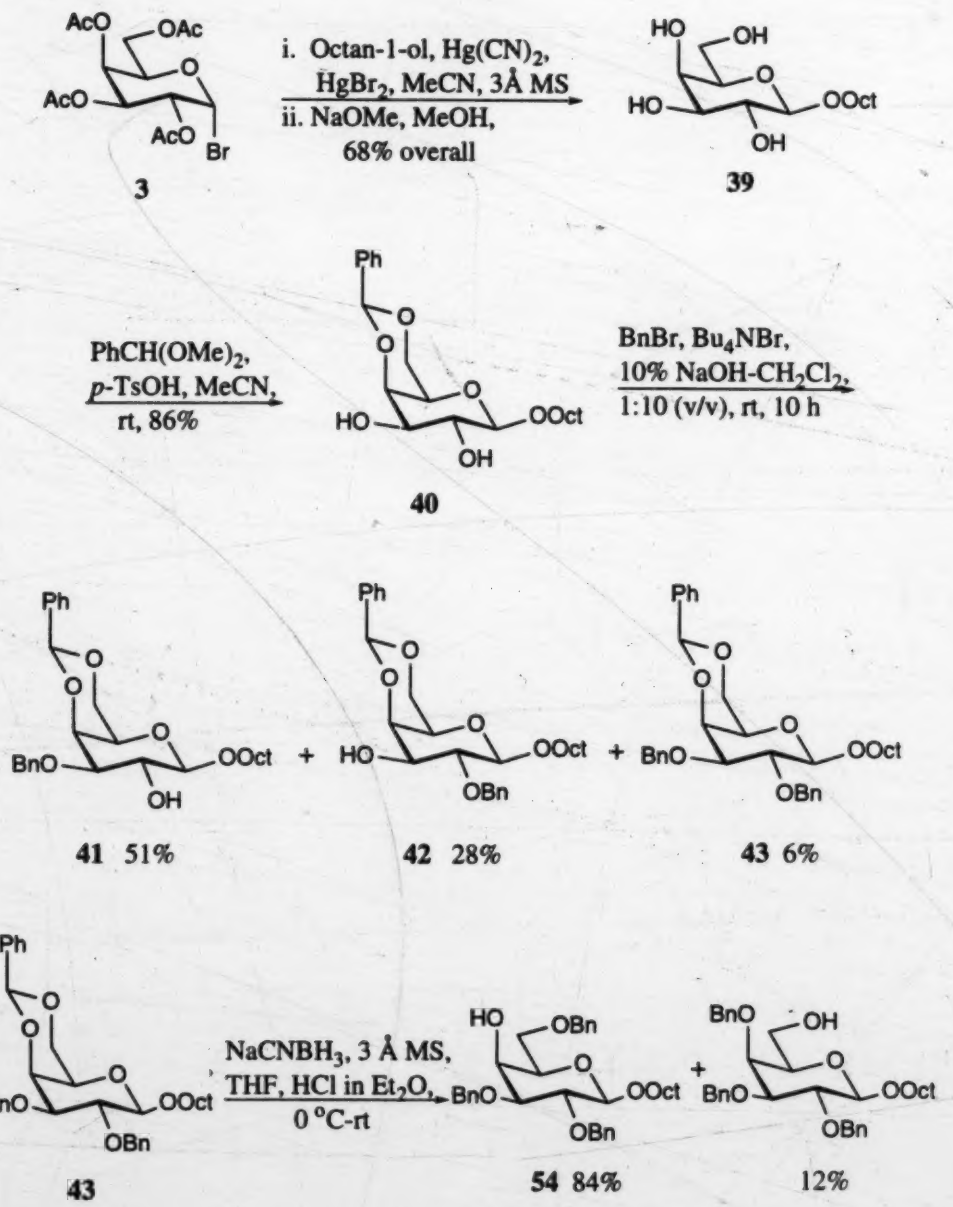
°C for 1 d followed by reaction with a mixture of pyridine and Ac_2O at 90 °C for 1 d to yield **34** in 76% overall yield (Scheme 2.8). 1-*O*-Acetylation of **34** using Ac_2O and BF_3 etherate did not give **36**, but resulted instead in debenzylidenation. The debenzylidenation of **34** with aq HOAc (80%) at 60 °C, followed by *O*-acetylation with Ac_2O and pyridine gave **35** (98% overall yield). Treatment of **35** with a mixture of TFA and CH_2Cl_2 (1:1) at 0 → 5 °C, followed by reaction with trichloroacetonitrile and DBU at 0 °C to rt for 3 h gave the trichloroacetimidate donor **37** in high yield (98%, two steps). Alternatively, reaction of **35** with Ac_2O and boron trifluoride etherate, followed by TMSOTf and TMSSMe in CH_2Cl_2 at rt for 3 d gave the thioglycoside donor **38** in low overall yield.

2.1.3. Preparation of Suitably Protected Mono-deoxygenated Octyl Galactopyranosyl Acceptors.

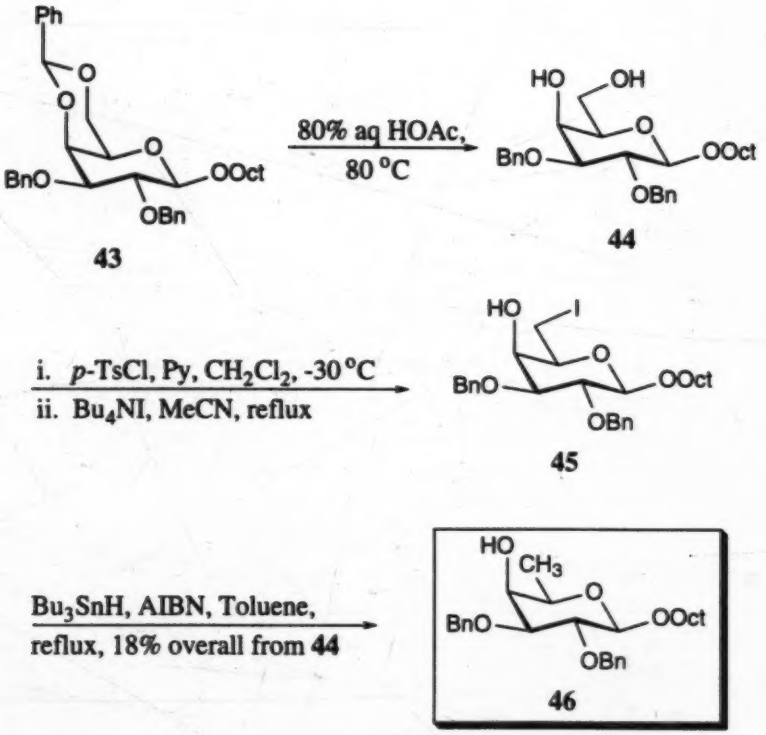
The synthesis of the suitably protected octyl galactopyranoside derivatives **41**, **42** and **43** is described in Scheme 2.9. Reaction of donor **3** with octan-1-ol in the presence of $Hg(CN)_2$, $HgBr_2$ and 3 Å molecular sieves in MeCN, followed by *O*-deacetylation with NaOMe in MeOH, gave **39** (68% overall yield). Benzylidenation of **39** then gave **40** in 86% yield. Monobenzylation of **40** under phase-transfer catalysis conditions [72] using BnBr in the presence of Bu_4NBr in a mixture of 10% aq NaOH and CH_2Cl_2 (1:10, v/v) with vigorous stirring overnight yielded the 3-*O*-benzyl product **41** (51%), the 2-*O*-benzyl product **42** (28%) and the 2,3-di-*O*-benzyl product **43** (6%).

The octyl 2,3-di-*O*-benzyl-6-deoxy-β-D-galactopyranoside acceptor **46** was prepared as shown in Scheme 2.10. The debenzylidenation of **43** in 80% aq HOAc at 80 °C for 3 h gave **44** (77%). Treatment of **44** with *p*-TsCl in dry pyridine and CH_2Cl_2 at -30 → -5 °C for 4 h, followed by Bu_4NI in refluxing dry MeCN overnight under Ar gave

crude **45** [73]. Deiodination of crude **45** with Bu_3SnH and AIBN in refluxing toluene overnight furnished the 6-deoxy galactopyranoside **46** (18%, overall yield from **44**).



Scheme 2.9: Preparation of partially protected octyl galactopyranosides.

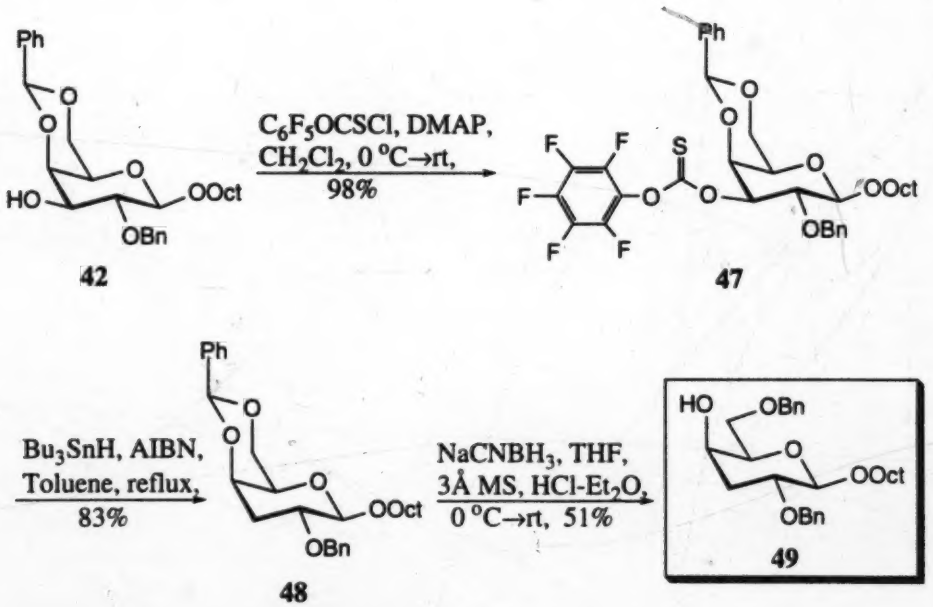


Scheme 2.10: Synthesis of the 6-deoxy glycosyl acceptor **46**.

The synthesis of the 3-deoxygenated octyl galactopyranoside acceptor **49** is shown in Scheme 2.11. Thiocarbonylation of **42** with pentafluorophenyl chlorothionoformate and DMAP in CH_2Cl_2 yielded **47** (98%). Deoxygenation of **47** with Bu_3SnH and AIBN in boiling toluene for 2 h gave **48** in 83% yield. Benzylidene ring opening of **48** with $NaCNBH_3$-HCl in the presence of 3 Å molecular sieves in THF at 0 °C to rt produced the 3-deoxygenated galactopyranoside acceptor **49** (51%).

The synthesis of the 2-deoxygenated octyl galactopyranoside acceptor **53** was performed as shown in scheme 2.12. Thiocarbonylation of **41** using the same method as

described for **42** gave **50** (80%). Deoxygenation of **50** then gave **51** in 94% yield (Scheme 2.12). Because the octyl aglycon in the 2-deoxy sugar was too labile it was cleaved during the benzylidene ring opening of **51** using $NaCNBH_3$-HCl in THF at 0 °C to give **52**. Alternatively, opening of the benzylidene ring of **51** under milder conditions,



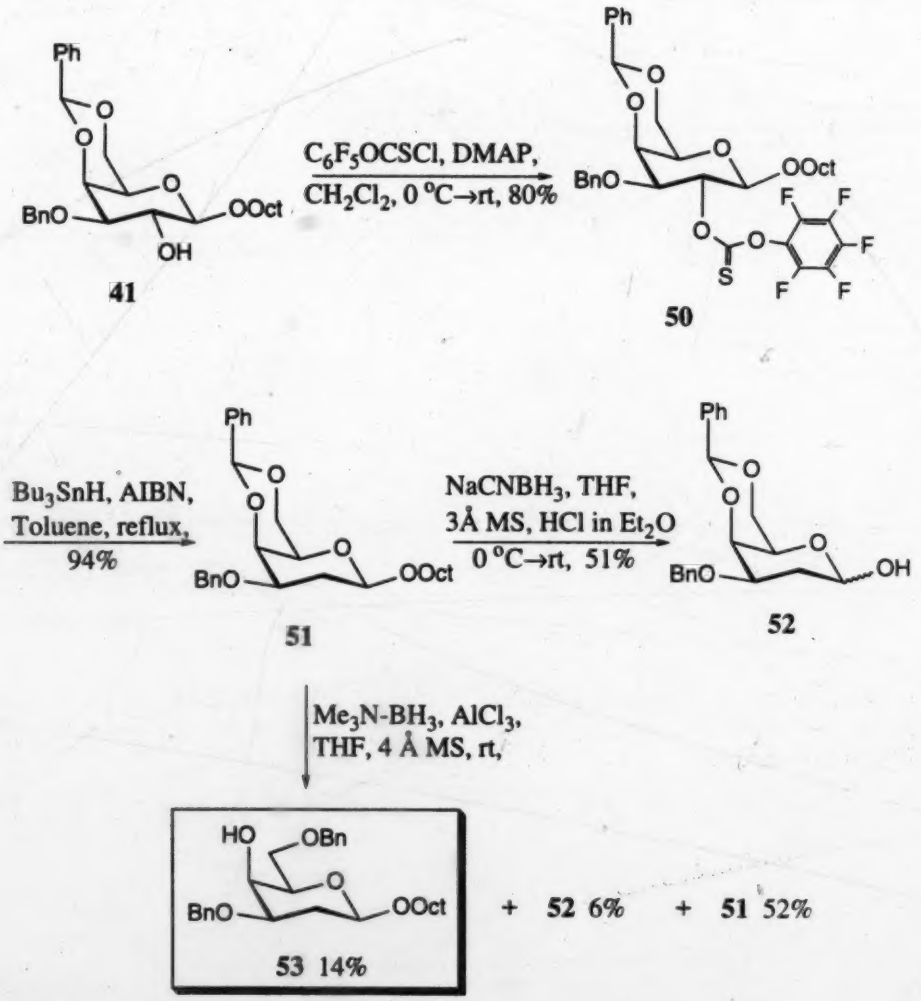
Scheme 2.11: Synthesis of the 3-deoxy glycosyl acceptor **49**.

using Me_3N-BH_3 and $AlCl_3$ in the presence of 4 Å molecular sieves in THF, gave **53** in low yield (14%, recovered 52% of starting material **51**) [74]. The yield could be increased using a longer reaction time, but this increased the extent of hydrolysis of the octyl group.

2.1.4. Glycosylation and Deprotection.

With the mono-deoxygenated galactosamine derivative donors **14**, **25**, **37** and mono-deoxygenated galactoside acceptors **46**, **49**, **53** in hand, the disaccharide-forming glycosylations were performed.

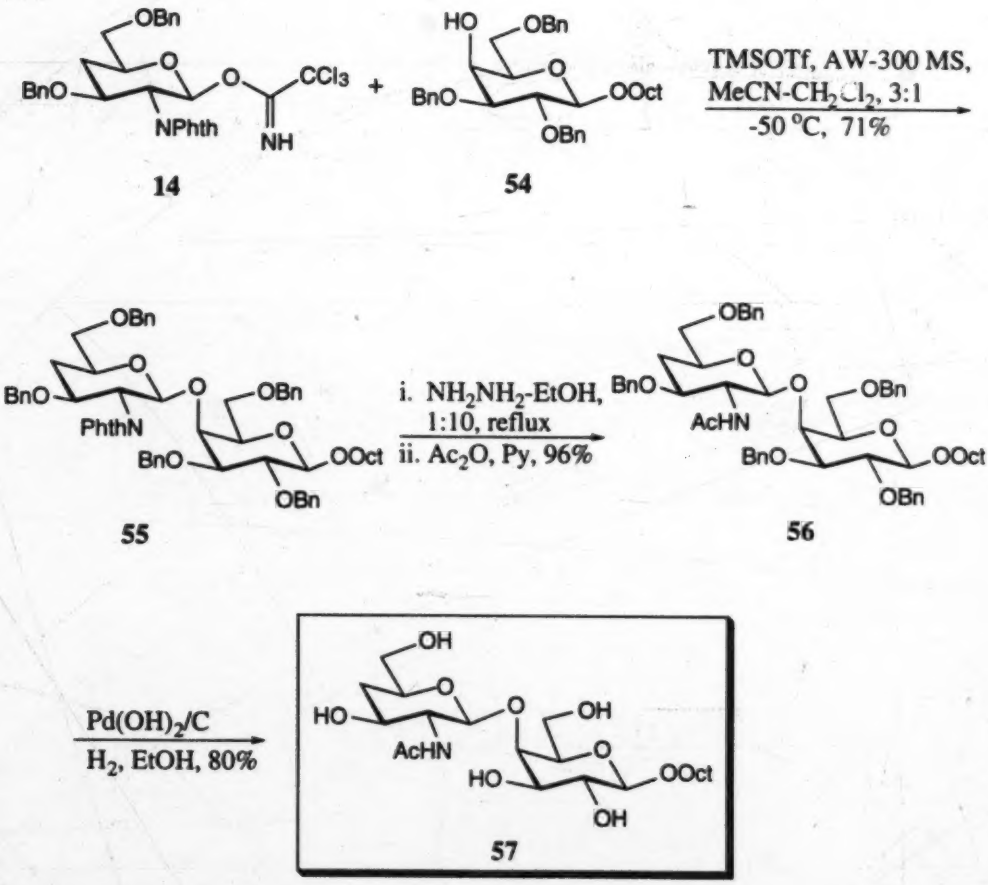
Glycosylation of the alcohol **54** with donor **14** using TMSOTf as the promoter in the presence of AW-300 molecular sieves in dry CH_3CN-CH_2Cl_2 (3:1) at -50 °C gave **55**



Scheme 2.12: Synthesis of the 2-deoxy glycosyl acceptor **53**.

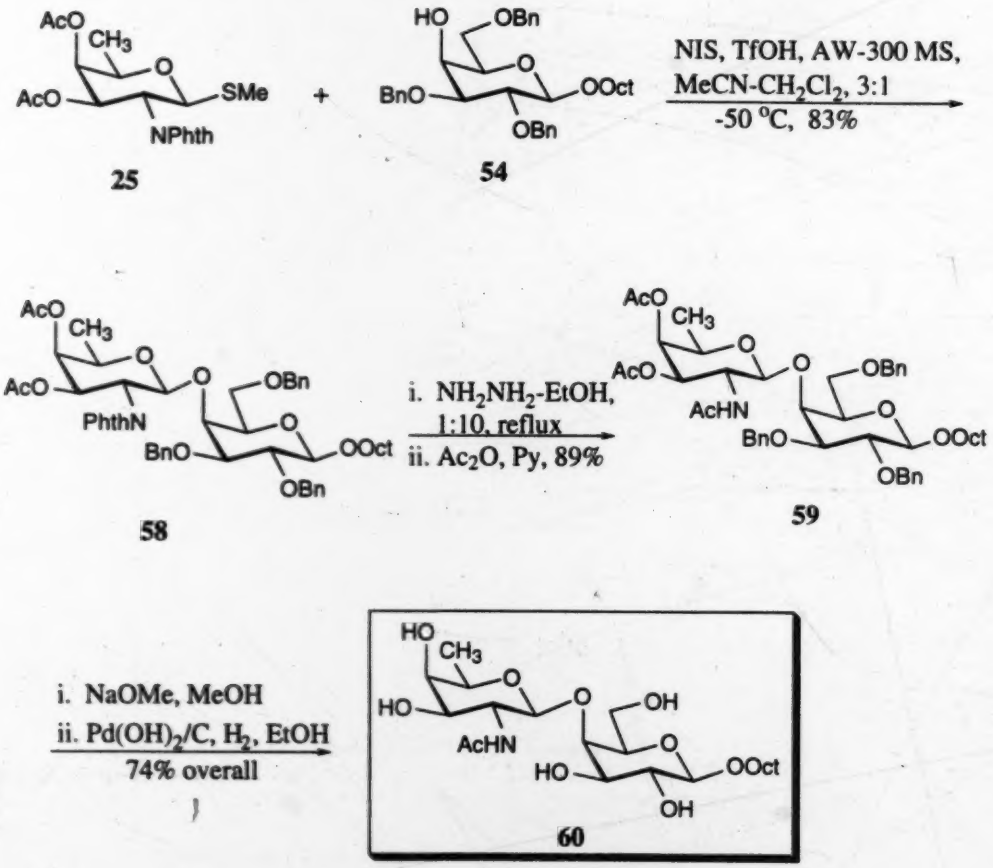
(71%) (Scheme 2.13) [75]. Hydrazinolysis of **55** in refluxing ethanol (1:10) followed by *N,O*-acetylation using Ac_2O and pyridine gave **56** in 96% overall yield [76]. Debenzylation of **56** with $Pd(OH)_2$-C (20%) (Pearlman's catalyst) [77] in ethanol (98%) furnished the target 4'-deoxy-disaccharide **57** in 80% yield.

N-Iodosuccinimide (NIS) and TfOH promoted the glycosylation of acceptor **54**

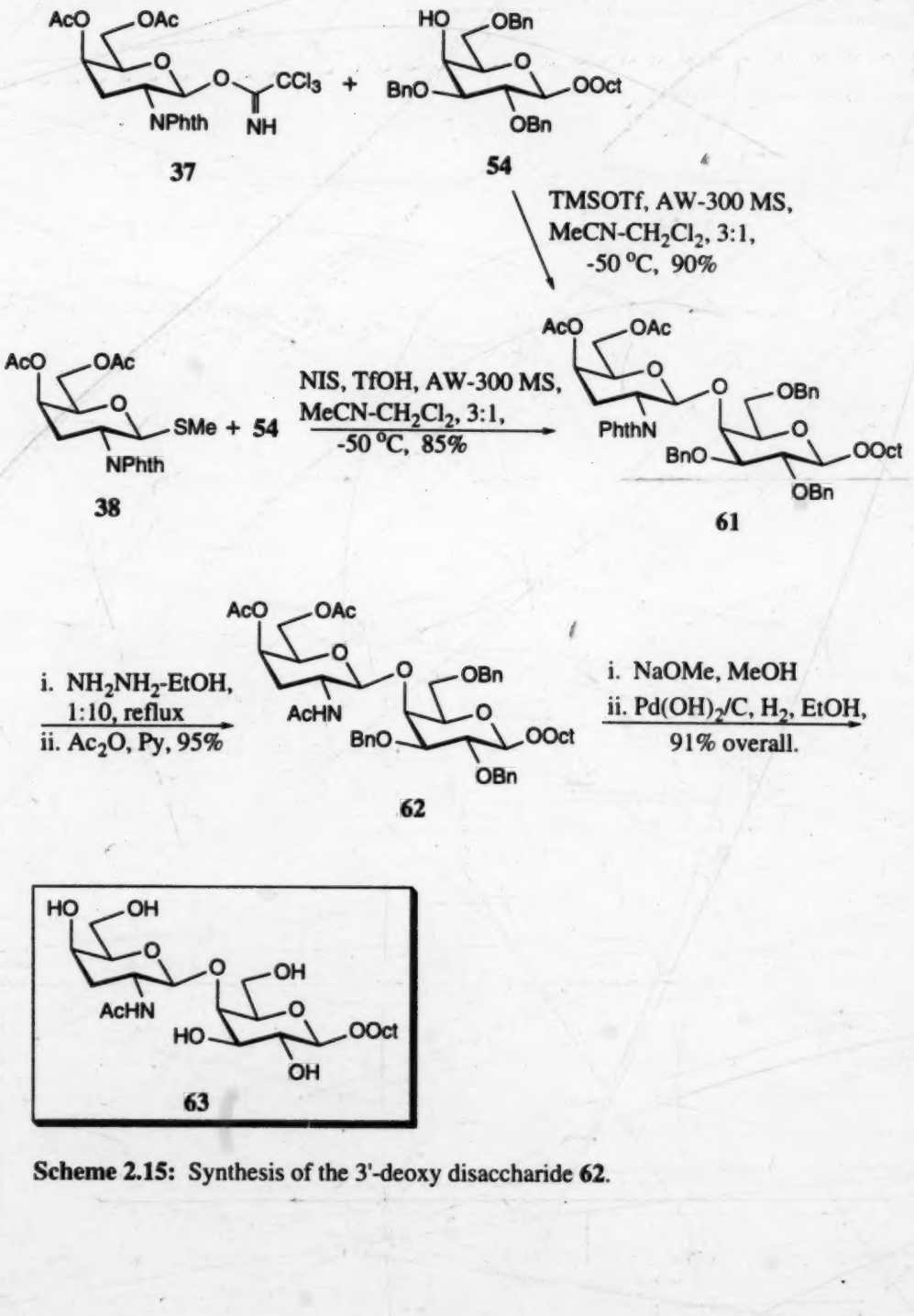


Scheme 2.13: Synthesis of the 4'-deoxy disaccharide **57**.

with the 6-deoxy galactosamine donor **25** in the presence of AW-300 molecular sieves in CH_3CN-CH_2Cl_2 at -50 °C to afford the disaccharide **58** (83%) [78] (Scheme 2.14). Hydrazinolysis of **58** followed by *N,O*-acetylation gave **59** in 89% overall yield. *O*-Deacetylation of **59** with NaOMe in MeOH followed by standard debenzylation gave the 6'-deoxy disaccharide **60** (74%).

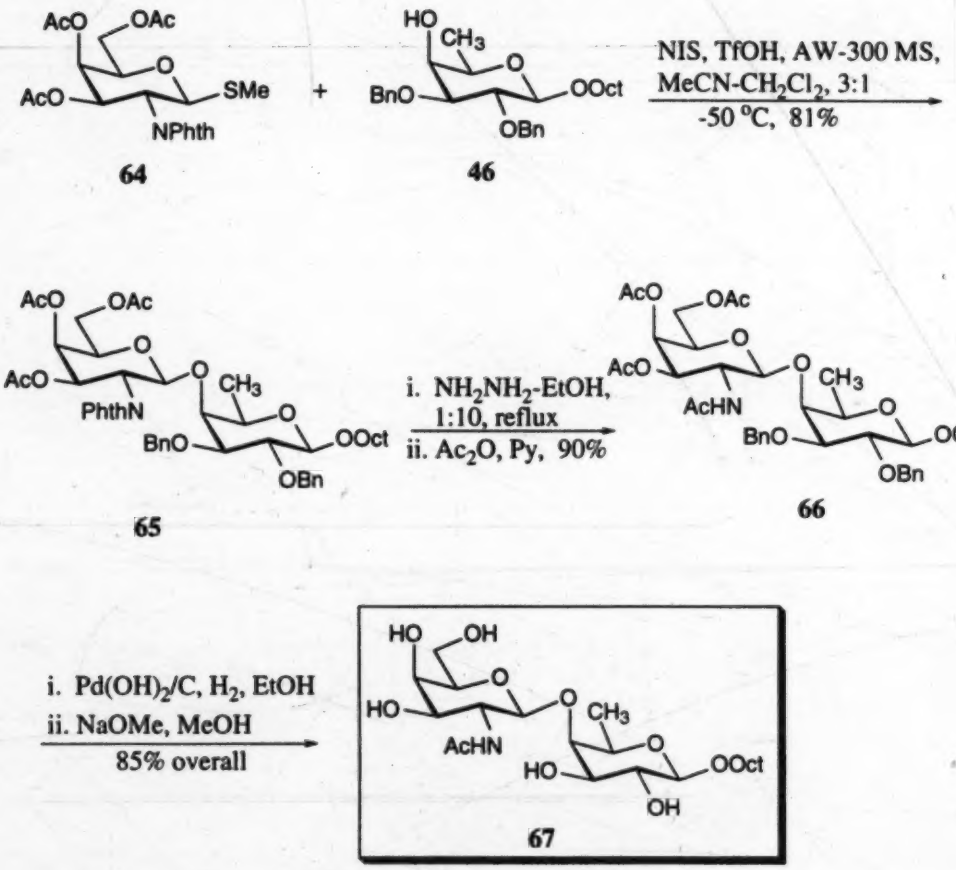


Scheme 2.14: Synthesis of the 6'-deoxy disaccharide **60**.



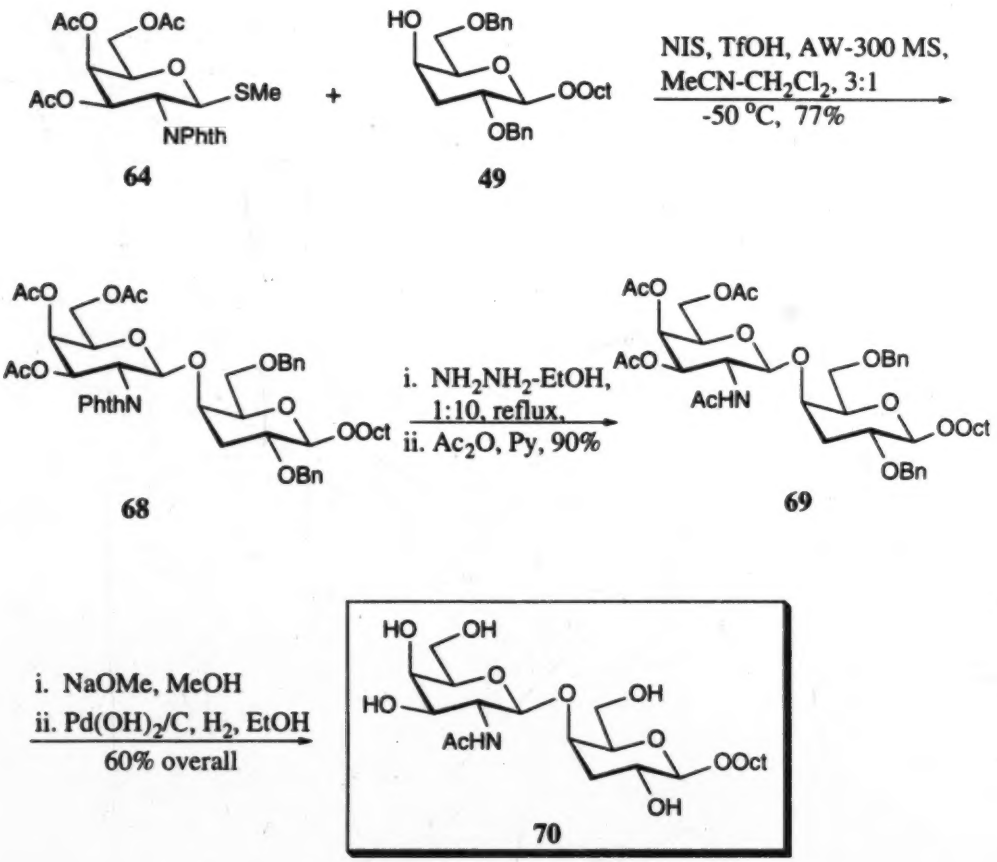
Scheme 2.15: Synthesis of the 3'-deoxy disaccharide **62**.

TMSOTf-promoted glycosylation of **37** and **54** in a mixture of CH_3CN-CH_2Cl_2 (3:1) and AW-300 molecular sieves at -50 °C yielded the disaccharide **61** (90%) (Scheme 2.15). Glycosylation of **35** with **54** using NIS and TfOH as the promoter system in a mixture of CH_3CN-CH_2Cl_2 (3:1) and AW-300 molecular sieves at -50 °C also gave **61** in 85% yield. Hydrazinolysis and *N,O*-acetylation of **61** gave **62** (95%). *O*-Deacetylation and debenzylation of **62** produced the 3'-deoxy-disaccharide **63** in 91% overall yield. The procedures and conditions for hydrazinolysis, *N,O*-acetylation, *O*-deacetylation and debenzylation were as described above.



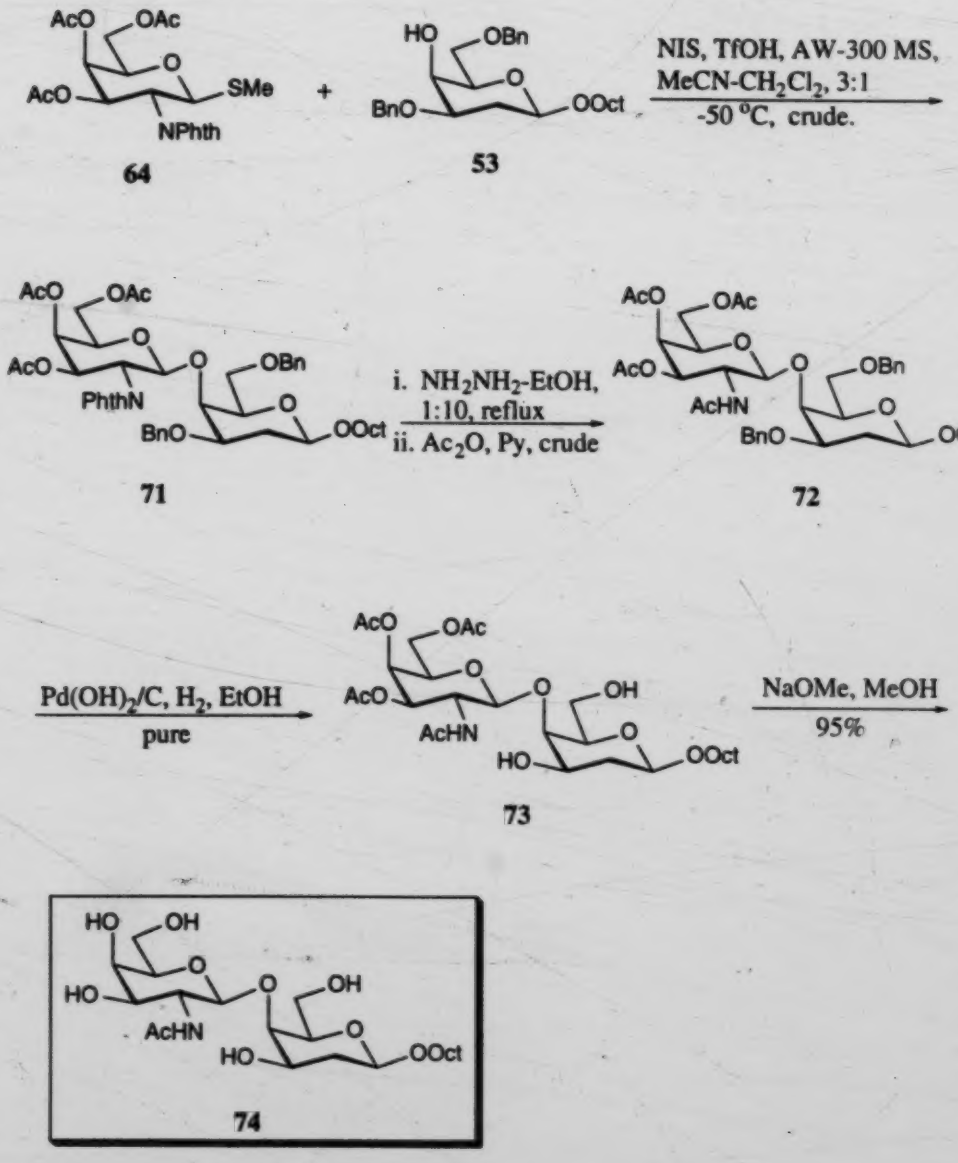
Scheme 2.16: Synthesis of the 6-deoxy disaccharide **67**.

Coupling of methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside (**64**) [79] and the octyl 6-deoxy galactopyranoside acceptor **46** with NIS-TfOH as the promoter in dry CH_3CN-CH_2Cl_2 at -50 °C gave disaccharide **65** in 81% yield (Scheme 2.16). Treatment of **65** with hydrazine monohydrate in refluxing 98% aq ethanol followed by acetylation gave **66** (90% in two steps). Hydrogenolysis of **66** using Pearlman's catalyst in ethanol followed by *O*-deacetylation with NaOMe in MeOH produced the 6-deoxy-disaccharide **67** in 85% overall yield.



Scheme 2.17: Synthesis of the 3-deoxy disaccharide **70**.

As for the synthesis of **69** (Scheme 2.17), condensation of the thio-galactopyranoside donor **64** with 3-deoxygenated octyl galactopyranoside acceptor **49** gave the disaccharide **68** (77%). Removal of the phthalimido group in **68** with hydrazine



Scheme 2.18: Synthesis of the 2-deoxy disaccharide **74**.

followed by *N,O*-acetylation gave **69** in 89% overall yield. Debenzylation and *O*-deacetylation of disaccharide **69** yielded the 3-deoxy-disaccharide **70** (60% overall yield).

Glycosylation of the 2-deoxy sugar acceptor **53** with the thiogalactopyranoside donor **64** using NIS-TfOH as a promoter gave crude **71** (Scheme 2.18). Compound **71** could not be purified by column chromatography. Hydrazinolysis of crude **71** followed by *N,O*-acetylation gave crude **72**. Purification of **72** using column chromatography was not successful. Debenzylation of crude **72** using $Pd(OH)_2/C$ in ethanol and column chromatography of the crude product finally provided the pure disaccharide **73**. *O*-Deacetylation of **73** with NaOMe in dry MeOH furnished the target 2-deoxy-disaccharide **74** in 94% yield.

2.2. Experimental Section.

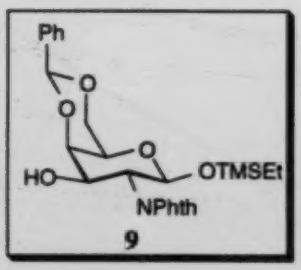
2.2.1. General Methods.

TLC was performed on Silica Gel 60-F_{254} (E. Merck) with detection by quenching of fluorescence, by charring with H_2SO_4 and/or by reaction with ninhydrin. Unless otherwise noted, column chromatography was performed on Silica Gel 60 (E. Merck, 40-63 μm). Beaded silica gel 6RS-8060 (Iatrobeads) was from Iatron Laboratories, Inc. (Japan). C_{18} Sep-Pak sample-preparation cartridges (reverse phase) were from Waters Associates (Mississauga, ON). Millex-GV (0.22 μm) filter units were from Millipore. Optical rotations were measured with a Perkin-Elmer 241 polarimeter at 22 ± 2 °C. ^1H NMR spectra were recorded at 300 MHz (Bruker AM 300), at 360 MHz (Bruker WM 360), or at 500 (Varian UNITY 500) in solutions of $CDCl_3$ (internal Me_4Si, δ 0), C_6D_6 (internal Me_4Si, δ 0), CD_3OD (δ 3.30), or D_2O (δ 4.82). Coupling constants can be ascribed a resolution of ± 0.5 Hz. ^{13}C NMR spectra were recorded at 75 MHz, at

90 MHz, or at 125 MHz, respectively, on the same instruments in $CDCl_3$ (δ 77.07), in D_2O (internal acetone, δ 31.07), and in CD_3OD (δ 49.0). FAB-mass spectra (FAB-MS) were obtained on a Kratos AEI-MS9 instrument. Electrospary ionization mass spectra (ESI-MS) were obtained from a Micromass ZabSpec Hybrid Sector-TOF instrument. Elemental analyses were carried out on a Carlo Erba EA1108 instrument by the departmental microanalytical laboratory.

2.2.2. Experimental.

2-(Trimethylsilyl)ethyl 4,6-O-bezylidene-2-deoxy-2-phthalimido-β-D-galactopyranoside (**9**).

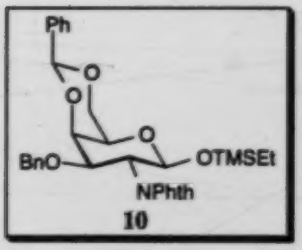


9

To a solution of **8** (1.85 g, 4.5 mmol) and benzaldehyde dimethyl acetal (1.35 ml, 9.0 mmol) in dried MeCN (20 mL), *p*-TsOH (16 mg, 0.9 mmol) was added with vigorous stirring at rt. TLC showed the absence of the starting material after 10 h reaction. The reaction mixture was neutralized with some Et_3N and then concentrated. The residue was purified with chromatography to give **9** (1.98 g, 88%). NMR ($CDCl_3$): ^1H: δ = 7.35-7.90 (m, 9 H, aromatics), 5.61 (s, 1 H, Ph*CH*O_2), 5.28 (d, 1H, J = 8.0 Hz, H-1), 4.50 (ddd, 1 H, J = 10.0, 10.0, 3.5 Hz, H-3), 4.43 (dd, 1 H, J = 10.0, 8.0 Hz, H-2), 4.41 (dd, 1 H, J = 12.5, 1.8 Hz, H-6a), 4.29 (dd, 1 H, J = 3.5, 1.8 Hz, H-4), 4.14 (dd, 1 H, J = 12.5, 1,8 Hz, H-6b), 4.00 (dq, 1 H, J = 9.5, 5.2 Hz, O*CH*CH_2Si), 3.65 (d, 1 H, J = 1.0, H-5), 3.52 (dt, 1 H, J = 6.8, 9.5 Hz, O*CH*CH_2Si), 0.93-0.70 (m, 2 H, OCH_2CH_2Si), and -0.9 (s, 9 H, TMS). ^{13}C: δ = 168.80, 168.32, 137.44, 133.98, 131.99, 129.35, 128.33,

126.56, 123.53, 123.05, 101.60, 97.85, 75.23, 69.30, 68.08, 66.75, 54.90, 17.78, and -1.48.

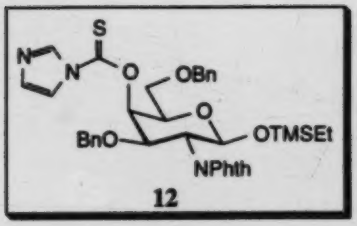
2-(Trimethylsilyl)ethyl 3-O-benzyl-4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-galactopyranoside (**10**).



10

NaH (48 mg, 80% in oil, 1.6 mmol) was added to a solution of compound **9** (385 mg, 0.8 mmol), Bu_4NI (590 mg, 1.6 mmol) and BnBr (191 μL, 1.6 mmol) in DMF (9 mL) 0 → 5 °C (ice bath) with stirring. The mixture was stirred for 10 h, at which time TLC showed the absence of starting material (R_f = 0.75, hexane-EtOAc, 1:1). MeOH (1 mL) was added to the reaction mixture to decompose the excess of NaH. The solution was diluted with EtOAc (100 mL), washed with brine (3 x 60 mL), dried with Na_2SO_4 and concentrated. The resulting residue was applied to a column (hexane-EtOAc, 3:1) to give product **10** (390 mg, 85%). NMR ($CDCl_3$): ^1H: δ = 7.0-7.9 (m, 11 H, aromatics), 5.55 (s, 1 H, Ph*CH*O_2), 5.2 (d, 1H, J = 8.5 Hz, H-1), 4.72 (dd, 1 H, J = 11.0, 8.5 Hz, H-2), 4.64 and 4.46 (2 d, 2 H, J = 11.5 Hz, Ph*CH*$_2$O), 4.42 (dd, 1 H, J = 11.0, 3.5 Hz, H-3), 4.36 (dd, 1 H, J = 12.0, 1.5 Hz, H-6a), 4.24 (d, 1 H, J = 3.5 Hz, H-4), 4.09 (dd, 1 H, J = 12.0, 1,5 Hz, H-6b), 3.98 (m, 1 H, O*CH*CH_2Si), 3.48 (m, 1 H, O*CH*CH_2Si), and 0.8 (m, 2 H, OCH_2CH_2Si). ^{13}C: δ = -1.5, 17.71, 52.27, 66.35, 66.71, 69.44, 70.98, 72.81, 74.09, 97.92, 101.17, 122.09, 123.51, 126.56, 127.59, 128.16, 131.84, 132.00, 133.70, 133.92, 137.84, 138.01, 167.58, and 168.79.

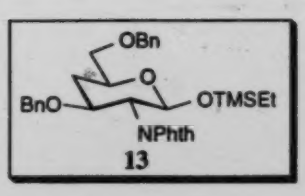
2-(Trimethylsilyl)ethyl 3,6-di-O-benzyl-2-deoxy-2-phthalimido-4-O-(1-thiocarbonylimidazol)-β-D-galactopyranoside (**12**).



12

A mixture of **11** (40 mg, 68 μmol), *N,N*-thiocarbonyldiimidazole (60 mg, 340 μmol) in dry DME (1.5 mL) was refluxed overnight and then concentrated. The residue was diluted with CH_2Cl_2 (20 mL), sequentially washed with aq HCl (1 M, 10 mL), water and satd $NaHCO_3$, dried with Na_2SO_4 and concentrated. The resulting residue was passed through a silica gel column (hexane-EtOAc, 1:1, R_f = 0.63) to give compound **12** (42 mg, 89%; $[\alpha]_D$ = + 94°, *c* = 2.3, in $CHCl_3$). NMR ($CDCl_3$): ^1H: δ = [8.60 (s, 1 H), 7.81 (s, 1 H), 7.40 (s, 1 H) imidazol], 8.0-6.95 (m, 14 H, aromatics), 6.60 (d, 1 H, J = 3.0 Hz, H-4), 5.23 (d, 1 H, J = 8.2 Hz, H-1), 4.61 and 4.28 (d, 2 H, J = 12.5 Hz, O*CH*$_2$Ph), 4.50 (s, 2 H, O*CH*$_2$Ph), 4,41 (dd, 1 H, J = 3.0, 11.0 Hz, H-3), 4.33 (dd, 1 H, J = 8.2, 11.0 Hz, H-2), 4.07 (bt, 1 H, J = 6.5 Hz, H-5), 4.92 (m, 1 H, O*CH*CH_2Si), 3.73-3.55 (m, 2 H, 2 x H-6), 3.54-3.43 (m, 1 H, O*CH*CH_2Sl), 0.8 (m, 2 H, OCH_2*CH*$_2$Si), and -0.15 (s, 9 H, $SiMe_3$). ^{13}C: δ = 184.45, 168.16, 167.35, 137.35, 136.99, 134.01, 133.77, 131.82, 131.65, 131.09, 128.47, 128.21, 128.10, 128.03, 127.80, 123.53, 123.16, 117.94, 98.17, 75.77, 73.89, 73.34, 72.45, 72.33, 67.88, 67.13, 53.50, 17.79, and -1.48. Anal. Calcd for $C_{37}H_{41}N_3SSi$: C, 63.75; H, 6.10; N, 5.94. Found: C, 63.75; H, 6.09; N, 5.93.

2-(Trimethylsilyl)ethyl 3,6-di-O-benzyl-2,4-dideoxy-2-phthlimido-β-D-xylopyranoside (**13**).

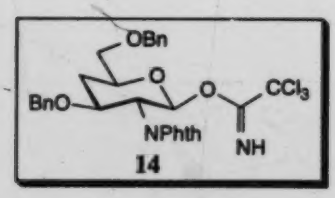


13

Bu_3SnH (45 μL, 170 μmol) in dry toluene (0.15 mL) and AIBN (catalytic amount) were added sequentially to a solution of compound **12** (60 mg, 85 μmmol) in dry toluene (1.5 mL) at rt under Ar. The mixture was refluxed overnight and concentrated. The residue was chromatographed (hexane-EtOAc, 3:1, R_f =

0.48) to give compound **13** (42 mg, 85%; $[\alpha]_D$ = + 24°, *c* = 0.6, in $CHCl_3$). NMR ($CDCl_3$): ^1H: δ = 7.9-6.9 (m, 14 H, aromatics), 5.15 (d, 1 H, J = 8.5 Hz, H-1), 4.64 (s, 2 H, O*CH*$_2$Ph), 4.60 and 4.30 (2 d, 2 H, J = 12.5 Hz, O*CH*$_2$Ph), 4.12 (dd, 1 H, J = 8.5, 10.0 Hz, H-2), 4.28 (bm, 1 H, H-5), 3.95 (m, 1 H, O*CH*CH_2Si), 3.73-3.54 (m, 2 H, 2 x H-6), 3.49 (m, 1 H, O*CH*CH_2Si), 2.32 (ddd, 1 H, J = 5.0, 12.5, 1.8 Hz, H-4e), 1.55 (bq, 1 H, J = 12.5 Hz, H-4a), 0.8 (m, 2 H, OCH_2*CH*$_2$Si), and -0.3 (s, 9 H, $SiMe_3$). ^{13}C: δ = 168.32, 138.13, 138.06, 133.72, 131.86, 128.38, 128.09, 127.65, 127.48, 127.38, 123.16, 98.02, 73.49, 72.93, 72.45, 71.20, 70.73, 66.61, 57.08, 34.35, 17.78, and -1.55. Anal. Calcd for $C_{33}H_{39}NO_6Si$: C, 69.08; H, 6.85; N, 2.44. Found: C, 68.90; H, 6.9; N, 2.64.

O-(3,6-Di-O-benzyl-2,4-dideoxy-2-phthalimido-β-D-xylopyranosyl) trichloroacetimidate (**14**).

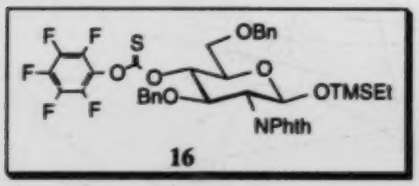


14

A solution of compound **13** (134 mg, 0.234 mmol) in dry CH_2Cl_2-TFA (1:1, 3 mL) was stirred at about 0 °C for 10 min. TLC verified the reaction had gone to completion. The reaction solution was concentrated, co-evaporated with a mixture of toluene and PrOAc (1:1, 2 x 10 mL) followed by co-evaporation with toluene (2 x 5 mL). Trichloroacetonitrile (236 µL, 2.34 mmol) and DBU (3.5 µL, 23.4 µmol) ware added to the solution of the residue in dry CH_2Cl_2 (4 mL) at 0 °C with stirring. The mixture was warmed to rt very slowly over 2 h and then concentrated. The resulting residue was chromatographed on a silica gel column (hexane-EtOAc, 4:1 containing 1% of Et_3N) to yield compound **14** (101 mg, 70%; R_f = 0.42 (hexane-EtOAc, 2:1; $[\alpha]_D$ = + 71°, *c* = 0.5, in CCl_4). NMR ($CDCl_3$): ^1H: δ = 8.50 (s, 1 H, *NH*), 7.80-7.05 (m, 14 H, aromatics), 6.40 (d, 1 H, J = 8.5 Hz, H-1), 4.61 (s, 2 H, O*CH*$_2$Ph), 4.62 and 4.34 (2 d, 2 H, J = 12.5 Hz, O*CH*$_2$Ph), 4.44 (ddd, 1 H, J = 11.0,

6.0, 5.0 Hz, H-3), 4.39 (dd, 1 H, J = 8.5, 11.0 Hz, H-2), 4.00 (dddd, 1 H, J = 12.0, 5.5, 5.5, 2.0 Hz, H-5), 3.71 (dd, 2 H, J = 10.0, 5.0 Hz, 2 x H-6), 2.35 (ddd, 1 H, J = 13.0, 4.5, 2.0 Hz, H-4e), and 1.65 (dd, 1 H, J = 12.0, 13.0 Hz, H-4a). ^{13}C: δ = 167.87, 161.00, 138.03, 137.99, 134.15, 133.99, 133.91, 131.66, 128.47, 128.24, 127.88, 127.79, 127.56, 123.36, 94.67, 73.52, 72.64, 72.46, 71.81, 71.12, 56.02, and 34.19. Anal. Cald for $C_{30}H_{27}Cl_3N_2O_6$: C, 58.31; H, 4.40; N, 4.53. Found: C, 58.17; H, 4.44; N, 4.49.

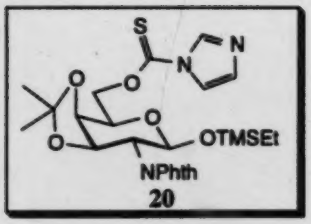
2-(Trimethylsilyl)ethyl 3,6-di-O-benzyl-2-deoxy-4-O-pentafluorophenoxythionocarbonyl-2-phthalimido-β-D-glucopyranoside (**16**).



16

Compound **16** (585 mg, 91%) was synthesized from **15** (460 mg, 0.8 mmol), pentafluorophenyl chlorothionoformate (1.0 mL, 5.0 mmol), and DMAP (976 mg, 8.0 mmol) as described for **30**. NMR ($CDCl_3$): ^1H: δ = 7.70-6.85 (m, 14 H, aromatics), 5.68 (dd, 1 H, J = 10.0, 8.7 Hz, H-4), 5.22 (d, 1 H, J = 8.5 Hz, H-1), 4.76 and 4.35 (2 d, 2 H,J = 12.5 Hz, O*CH*$_2$Ph), 4.60 (s, 2 H, O*CH*$_2$Ph), 4,58 (dd, 1 H, J = 10.0, 8.7 Hz, H-3), 4.30 (dd, 1 H, J = 8.5, 10.7 Hz, H-2), 3.95 (m, 2 H, H-5 and O*CH*CH_2Si), 3.78 (dd, 1 H, J = 10.7, 2.9 Hz, H-6a), 3.68 (dd, 1 H, J = 10.7, 5.9 Hz, H-6b), 3.5 (m, 1 H, O*CH*CH_2Si), 0.8 (m, 2 H, OCH$_2$*CH*$_2$Si), and -0.12 (s, 9 H, $SiMe_3$).

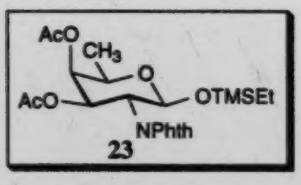
2-(Trimethylsilyl)ethyl 2-deoxy-3,4-O-isopropylidene-2-phthalimido-6-O-(1-thiocarbonylimidazol)-β-D-galactopyranoside (**20**).



20

A solution of compound **19** (459.5 mg, 1.02 mmol), *N,N*-thiocarbonyldiimidazole (910.9 mg, 5.11 mmol) in dry DME (20 mL) was refluxed overnight, concentrated, diluted with CH_2Cl_2, sequentially washed with aq HCl (1 M), water, satd $NaHCO_3$, dried (Na_2SO_4), and concentrated. The residue was chromatographed (hexane-EtOAc, 3:2) to give compound **20** (540 mg, 94%; R_f = 0.63, hexane-EtOAc, 1:1; $[\alpha]_D$ = + 27°, *c* = 2.2, in $CHCl_3$). NMR ($CDCl_3$): ^1H: δ = [8.35 (bd, 1 H, J = 1.0 Hz), 7.61 (t, 1 H, J = 1.0 Hz), 7.01 (m, 1 H), imidazole], 7.86-7.65 (m, 4 H, aromatic), 5.11 (d, 1 H, J = 8.9 Hz, H-6a), 5.05 (dd, 1 H, J = 4.0, 12.0 Hz, H-6b), 4.93 (dd, 1 H, J = 8.0, 12.0 Hz, H-1), 4.80 (dd, 1 H, J = 9.0, 5.0 Hz, H-3), 4.40 (m, 1 H, H-5), 4.27 (bt, J = 9.0 Hz, H-2), 4.25 (dd, 1 H, J = 2.0, 5.0 Hz, H-4), 3.90 and 3.47 (2 m, 2 H, OCH_2CH_2Si), 1.63 and 1.32 (2 s, 6 H, $O_2C(CH_3)_2$), 0.79 (m, 2 H, OCH_2CH_2Si), and -0.2 (s, 9 H, $SiMe_3$). ^{13}C: δ = 183.84, 171.15, 134.11, 131.92, 130.93, 123.46, 117.84, 111.09, 97.62, 74.35, 73.34, 71.82, 70.47, 67.08, 60.40, 54.88, 27.89, 26.56, 21.05, 17.75, 14.22, and -1.56. Anal. Calcd for $C_{26}H_{33}N_3O_7SSi$: C, 59.79; H, 5.94; N, 7.51. Found: C, 59.52; H, 6.04; N, 7.43.

2-(Trimethylsilyl)ethyl 3,4-di-O-acetyl-2,6-dideoxy-2-phthalimido-β-D-galactopyranoside (**23**).

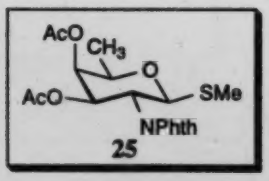


23

To a solution of compound **20** (510 mg, 0.91 mmol) in dry toluene (20 mL), Bu_3SnH (2 mL, 7.3 mmol) in dry toluene (2 mL) was added followed by AIBN (catalytic amount). The mixture was refluxed overnight and then concentrated. Column chromatography of the residue (hexane-EtOAc, 3:1) gave crude compound **22**. The crude **22** was dissolved in aq HOAc (80%, 50 mL) at rt and then

heated to 40 °C slowly. After 2 h, the solution was concentrated and co-evaporated with toluene three times. The resulting residue was dissolved in pyridine-Ac_2O (2:1, 50 mL) at rt overnight with stirring. The reaction solution was concentrated and co-evaporated with toluene three times. The residue was passed through a silica gel column (hexane-EtOAc, 3:1) to give compound **23** (280 mg, 64%; R_f = 0.72, hexane-EtOAc, 1:1; $[\alpha]_D$ = - 3°, *c* = 0.6, in $CHCl_3$). NMR ($CDCl_3$): ^1H: δ = 7.85-7.70 (m, 4 H, aromatic), 5.75 (dd, 1H, J = 11.5, 3.5 Hz, H-3), 5.31 (dd, 1 H, J = 3.5, 0.8 Hz, H-4), 5.28 (d, 1 H, J = 8.4 Hz, H-1), 4.50 (dd, 1 H, J = 8.4, 11.5 Hz, H-2), 3.97 (qd, 1 H, J = 6.5, 0.8 Hz, H-5), 3.95 (m, 1 H, O*CH*CH_2Si), 3.47 (dt, 1 H, J = 7.0, 10.0 Hz, O*CH*CH_2Si), 2.20 and 1.85 (2 s, 6 H, 2 x OAc), 1.27 (d, 3 H, J = 6.5 Hz, 3 x H-6), 0,78 (m, 2 H, OCH_2*CH_2*Si) and -0.17 (s, 9 H, $SiMe_3$). ^{13}C: δ = 251.58, 170.86, 134.21, 131.70, 123.58, 123.44, 97.78, 70.13, 69.20, 68.70, 67.23, 51.59, 33.32, 20.82, 20.61, 17.85, 16.32, and -1.52. Anal. Calcd for $C_{23}H_{31}NO_8Si$: C, 57.84; H, 6.54; N, 2.93. Found: C, 57.80; H, 6.60; N, 2.90.

Methyl 3,4-di-O-acetyl-2,6-dideoxy-2-phthalimido-1-thio-β-D-galactopyranoside (**25**).

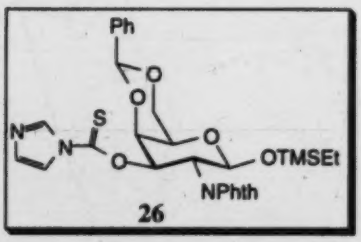


25

To a solution of **23** (61 mg, 128 μmol) in dry CH_2Cl_2 (1.5 mL) was added Ac_2O (36 μL, 384 μmol) and $BF_3{\cdot}Et_2O$ (20 μL, 145 μmol) under Ar at rt. After 3 h, TLC indicated the reaction had gone to completion. The mixture was diluted with CH_2Cl_2, washed with satd $NaHCO_3$, dried with Na_2SO_4, filtered, concentrated and co-evaporated with toluene three times. A solution of the residue, TMSOTf (25 μL, 128 μmol) and TMSSMe (65 μL, 512 μmol) in dry CH_2Cl_2 (1.5 mL) was stirred 2 d under Ar and then diluted with CH_2Cl_2, washed with satd $NaHCO_3$, dried with Na_2SO_4, filtered and concentrated. The resulting residue was passed through a silica gel column (hexane-

EtOAc, 5:2) to yield compound **25** (50 mg, 96%; R_f = 0.29, hexane-EtOAc, 2:1; $[\alpha]_D$ = -3°, *c* = 0.7, in $CHCl_3$). NMR ($CDCl_3$): ^1H: δ = 7.95-7.70 (m, 4 H, aromatic), 5.87 (dd, 1 H, J = 10.8, 3.1 Hz, H-3), 5.37 (dd, 1 H, J = 3.1, 1.0 Hz, H-4), 5.32 (d, 1 H, J = 10.4 Hz, H-1), 4.62 (bt, 1 H, J = 10.4, 10.8 Hz, H-2), 4.04 (qd, 1 H, J = 6.5, 1.0 Hz, H-5), 2.25 (s, 3 H, SMe), 2.20 and 1.80 (2 s, 6 H, 2 x OAc), and 1.23 (d, 3 H, J = 6.5 Hz, 3 x H-6). ^{13}C: δ = 170.62, 169.76, 167.96, 167.59, 134.34, 123.23, 131.65, 131.30, 123.65, 123.58, 80.77, 73.28, 70.16, 69.11, 49.61, 20.71, 20.54, 16.57, and 11.64. Anal. Calcd for $C_{19}H_{21}NO_7S$: C, 56.01; H, 5.20; N, 3.44. Found: C, 55.97; H, 5.23; N, 3.40.

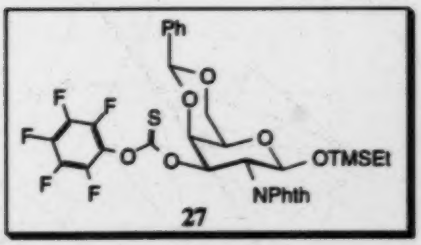
2-(Trimethylsilyl)ethyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-3-O-(1-thiocarbonylimidazol)-β-D-galactopyranoside (**26**).



26

Compound **26** (354 mg, 98%) was synthesized from **9** (297 mg, 0.6 mmol) and *N,N*-thiocarbonyldiimidazole (590 mg, 3.0 mmol) as described for **12**. NMR ($CDCl_3$): ^1H: δ = 8.40-6.80 (m, 12 H, aromatics), 6.63 (dd, 1 H, J = 3.8, 11.5 Hz, H-3), 5.58 (s, 2 H, O*CH*$_2$Ph),.5.48 (d, 1 H, J = 8.5 Hz, H-1), 5.00 (dd, 1 H, J = 8.5, 11.5 Hz, H-2), 4.79 (d, 1 H, J = 3.8 Hz, H-4), 4.45 (dd, 1 H, J = 1.5, 12.5 Hz, H-6a), 4.04 (m, 1 H, O*CH*CH$_2$Si), 4.18 (dd, 1 H, J = 1.5, 12.5 Hz, H-6b), 3.76 (d, 1 H, J = 1.5 Hz, H-5), 3.54 (m, 1 H, O*CH*CH$_2$Si), 0.8 (m, 2 H, OCH$_2$*CH*$_2$Si), and -0.15 (s, 9 H, SiMe$_3$). ^{13}C: δ = 182.68, 168.55, 167.45, 137.25, 134.40, 134.33, 131.60, 131.09, 129.12, 128.26, 126.18, 123.89, 123.42, 117.79, 100.92, 97.34, 77.30, 71.86, 69.11, 66.97, 66.26, 51.02, 17.79, and -1.49. Anal. Calcd for $C_{37}H_{41}N_3SSi$: C, 59.29; H, 5.47; N, 6.91; Found: C, 59.12; H, 5.51; N, 6.82.

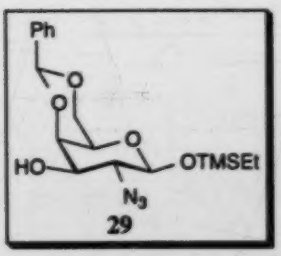
2-(Trimethylsilyl)ethyl 4,6-O-benzylidene-2-deoxy-3-O-pentafluorophenoxythionocarbonyl-2-phthalimido-β-D-galactopyranoside (**27**).



27

Compound **27** (70 mg, 96%) was synthesized from **9** (50 mg, 0.1 mmol), pentafluorophenyl chlorothionoformate (0.1 mL, 0.6 mmol), and DMAP (73 mg, 0.6 mmol) as described for **30**. NMR ($CDCl_3$): ^1H: δ = 8.0-7.4 (m, 9 H, aromatics), 6.15 (dd, 1 H, J = 3.5, 11.5 Hz, H-3), 5.62 (s, 2 H, O*CH*$_2$Ph),.5.39 (d, 1 H, J = 8.5 Hz, H-1), 4.86 (dd, 1 H, J = 8.5, 11.5 Hz, H-2), 4.76 (d, 1 H, J = 3.5 Hz, H-4), 4.41 (dd, 1 H, J = 1.5, 12.5 Hz, H-6a), 4.00 (m, 1 H, O*CH*CH_2Si), 4.27-4.13 (m, 2 H, H-5 and H-6b), 3.52 (m, 1 H, O*CH*CH_2Si), 0.8 (m, 2 H, OCH_2*CH*$_2$Si), and -0.15 (s, 9 H, $SiMe_3$).

2-(Trimethylsilyl)ethyl 2-azido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranoside (**29**).

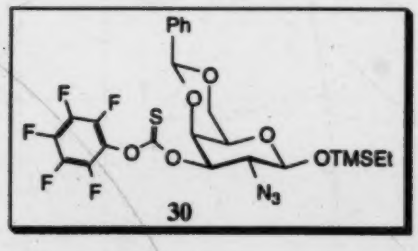


29

To a solution of **7** (2.0 g, 6.7 mmol) and benzaldehyde dimethyl acetal (2.0, 13.1 mmol) in dried MeCN (35 mL), *p*-TsOH (28 mg, 0.16 mmol) was added with vigorous stirring at rt. TLC showed the absence of the starting material after 16 h reaction. The reaction mixture was neutralized with some Et_3N and then concentrated. A solution of the residue in CH_2Cl_2 (40 mL) was washed with H_2O (2 x 10 mL), dried with $MgSO_4$, filtered and evaporated to dryness. The resulting residue was purified with chromatography to give **29** (2.26 g, 77%; R_f = 0.19, hexane-EtOAc, 2:1). NMR ($CDCl_3$): ^1H: δ = 7.54-7.36 (m, 5 H, aromatic), 5.58 (s, 1 H, O_2*CH*Ph), 4.31 (d, 1 H, J = 7.5 Hz, H-1), 4.36 (dd, 1 H, J =

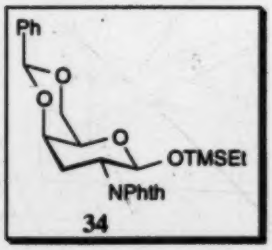
12.5, 1.0 Hz, H-6a), 4.18 (dd, 3.5, 1.0 Hz, H-4), 4.08 (dd, 1 H, J = 12.5, 1.5 Hz, H-6b), 4.06 (dd, 1 H, J = 10.0, 7.5 Hz, H-2), 3.56 (ddd, 1 H, J = 10.0, 10.0, 3.5 Hz, H-3), 3.65 and 3.57 (2 m, 2 H, OCH_2CH_2Si), 3.45 (bd, 1 H, J = 1.0 Hz, H-5), 1.06 (m, 2 H, OCH_2CH_2Si), and 0.04 (s, 9 H, $SiMe_3$).

2-(Trimethylsilyl)ethyl 2-azido-4,6-O-benzylidene-2-deoxy-3-O-pentafluorophenoxy-thionocarbonyl-β-D-galactopyranoside (**30**).



Pentafluorophenyl chlorothionoformate (2 mL, 12.7 mmol) was added with stirring to a solution of **29** (1.0 g, 1.54 mmol) in dry CH_2Cl_2 (10 mL) containing DMAP (2.0 g, 25.4 mmol) at 0 °C. The reaction mixture was allowed to increase to rt. After 15 h, the mixture was diluted with CH_2Cl_2, sequentially washed with 0.5% HCl, water, satd $NaHCO_3$ and water, dried with Na_2SO_4 and concentrated. The resulting residue was purified by column chromatography (hexane-EtOAc, 5:1) to afford compound **30** (1.43 g, 91%; R_f = 0.58, hexane-EtOAc, 3:1; $[\alpha]_D$ = + 26°, *c* = 0.7, in $CHCl_3$). NMR ($CDCl_3$): ^1H: δ = 7.55-7.30 (m, 5 H, aromatic), 5.58 (s, 1 H, O_2CHPh), 5.14 (dd, 1 H, J = 3.8, 10.8 Hz, H-3), 4.62 (dd, 1 H, J = 3.8, 0.8 Hz, H-4), 4.51 (d, 1 H, J = 8.0 Hz, H-1), 4.39 (dd, 1 H, J = 12.5, 1.5 Hz, H-6a), 4.10 (dd, 1 H, J = 12.5, 1.5 Hz, H-6b), 4.08 (dd, 1 H, J = 10.8, 8.0 Hz, H-2), 4.10 and 3.65 (2 m, 2 H, OCH_2CH_2Si), 3.51 (bd, 1 H, J = 0.8 Hz, H-5), 1.05 (m, 2 H, OCH_2CH_2Si), and 0.05 (s, 9 H, $SiMe_3$). ^{13}C: δ = 191.10, 142.89, 139.52, 138.50, 137.25, 136.33, 129.19, 128.24, 126.22, 101.60, 101.02, 82.97, 71.33, 68.95, 67.99, 66.04, 60.35, 18.22, and -1.40. Anal. Calcd. for $C_{25}H_{27}F_5N_3O_6SSi$: C, 48.46; H, 4.23; N, 6.78. Found: C, 48.51; H, 4.16; N, 6.81.

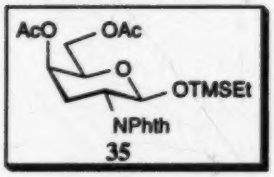
2-(Trimethylsilyl)ethyl 4,6-O-benzylidene-2,3-dideoxy-2-phthalimido-β-D-xylopyranoside (**34**).



34

To a solution of compound **30** (1.305 g, 2.1 mmol) in dry toluene (120 mL), Bu_3SnH (5.4 mL, 20 mmol) and AIBN (160 mg, 1 mmol) were added under Ar at rt. The mixture was refluxed overnight at 120 °C (oil bath temperature) and then concentrated. The residue was chromatographed (hexane-EtOAc, 3:1 to CH_2Cl_2-MeOH, 40:1) to give crude **33** (R_f = 0.20, CH_2Cl_2-MeOH, 20:1). Phthalic anhydride (183 mg, 1.2 mmol) was added to a solution of crude **33** in pyridine (8 mL). The solution was heated to 70 °C for 20 min and Et_3N (83 µL, 0.6 mmol) was added. The mixture was kept at this temperature overnight and then concentrated. The residue was taken in pyridine-Ac_2O (2:1, 20 mL) and heated to 90 °C for 3 h. The solution was concentrated and co-evaporated with toluene. The resulting residue was applied to a silica gel column (hexane-EtOAc, 4:1) to yield compound **34** (298 mg, 29% overall yield; R_f = 0.27, hexane-EtOAc, 3:1; $[\alpha]_D$ = - 24°, c = 0.4, in $CHCl_3$). NMR ($CDCl_3$): ^1H: δ = 7.90-7.30 (m, 9 H, aromatics), 5.58 (s, 1 H, O_2CHPh), 5.28 (d, 1 H, J = 8.5 Hz, H-1), 4.64 (ddd, 1 H, J = 13.0, 8.5, 4.5 Hz, H-2), 4.36 (dd, 1 H, J = 12.5, 1.0 Hz, H-6a), 4.13 (dd, 1 H, J = 12.5, 2.0 Hz, H-6b), 4.14 (bs, 1 H, H-4), 4.00 (ddd, 1 H, J = 11.0, 10.0, 5.2 Hz, O$CH$$CH_2$Si), 3.67 (bd, 1 H, J = 1.5 Hz, H-5), 3.49 (dt, 1 H, J = 6.8, 11.0 Hz, O$CH$$CH_2$Si), 2.74 (ddd, 1 H, J = 13.0, 13.5, 4.5 Hz, H-3a), 2.17 (ddd, 1 H, J = 13.5, 4.5, 2.5 Hz, H-3e), 0.9 (m, 2 H, OCH_2CH_2Si), and -0.08 (s, 9 H, $SiMe_3$). ^{13}C: δ = 137.99, 133.97, 131.98, 129.04, 128.24, 126.52, 123.24, 101.36, 99.33, 72.54, 69.67, 69.36, 66.16, 47.52, 31.93, 17.78, and -1.47. Anal. Calcd. for $C_{26}H_{31}O_6NSi$: C, 64.84; H, 6.49; N, 2.91. Found: C, 64.72; H, 6.49; N, 2.88.

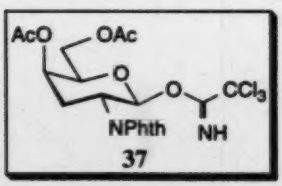
2-(Trimethylsilyl)ethyl 4,6-di-O-acetyl-2,3-dideoxy-2-phthalimido-β-D-xylopyranoside (**35**).



35

A solution of compound **34** (109 mg, 0.23 mmol) in aq HOAc (80%, 6 mL) was stirred at 60 °C overnight and then concentrated and co-evaporated with toluene three times. A solution of the residue in pyridine-Ac_2O (2:1, 10 mL) was kept overnight at rt and then evaporated to dryness and co-evaporated with toluene. The residue was purified with a silica gel column (hexane-EtOAc, 4:1) to yield compound **35** (106 mg, 98%; R_f = 0.67, hexane-EtOAc, 2:1; $[\alpha]_D$ = - 38°, *c* = 0.8, in CCl_4). NMR ($CDCl_3$): ^1H: δ = 7.86-7.68 (m, 4 H, aromatic), 5.30 (d, 1 H, J = 8.5 Hz, H-1), 5.11 (bs, 1 H, H-4), 4.45 (ddd, 1 H, J = 12.0, 8.5, 4.5 Hz, H-2), 4.20 (m, 2 H, 2 x H-6), 4.05 (td, 1 H, J = 6.5, 1.5 Hz, H-5), 3.85 (dq, 1 H, J = 10.0, 5.0 Hz, O*CH*CH_2Si), 3.52 (dt, 1 H, J = 7.0, 10.0 Hz, O*CH*CH_2Si), 2.65 (ddd, 1 H, J = 14.5, 12.0, 3.0 Hz, H-3a), 2.10 (ddd, 1 H, J = 14.5, 4.5, 3.0 Hz, H-3e), 2.16 and 2.07 (2 S, 6 H, 2 x OAc), 0.8 (m, 2 H, OCH_2*CH_2*Si), and -0.15 (s, 9 H, $SiMe_3$). ^{13}C: δ = 170.60, 170.50, 168.06, 134.13, 131.84, 123.36, 99.37, 74.09, 67.07, 66.77, 62.45, 47.74, 31.14, 21.13, 20.78, 17.82, and -1.52. Anal. Calcd. for $C_{23}H_{31}NO_8Si$: C, 57.84; H, 6.54; N, 2.93. Found: C, 57.73; H, 6.60; N, 2.80.

O-(4,6-di-O-acetyl-2,3-dideoxy-2-phthalimido-β-D-xylopyranosyl) trichloroacetimidate (**37**).

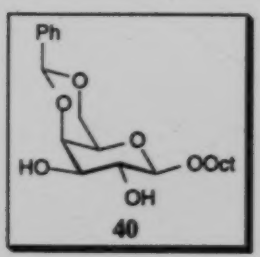


37

Compound **35** (100 mg, 0.21 mmol) was dissolved in dry CH_2Cl_2 and CF_3CO_2H (4:5, 4.5 mL) at 0 °C. After 2 h, TLC indicated the reaction had gone to completion (R_f = 0.31, hexane-EtOAc, 1:1). The solution was concentrated, co-evaporated twice with a mixture of toluene and PrOAc (1:1) and then once with toluene. To the solution of the residue in dry CH_2Cl_2 (2 mL), trichloroacetonitrile (212 µL, 2.1 mmol) and DBU (6.0 µL, 42 µmol) were added at 0 °C. The reaction solution was

allowed to increase to rt slowly. After 2 h, the solution was evaporated to dryness. The residue was purified by chromatography (2:1, hexane-EtOAc containing 1% Et_3N) to yield compound **37** (105 mg, 98%; R_f = 0.43, hexane-EtOAc, 1:1; $[\alpha]_D$ = + 9°, *c* = 0.7, in $CHCl_3$). NMR ($CDCl_3$): ^1H: δ = 8.58 (s, 1 H, *NH*), 7.86-7.68 (m, 4 H, aromatic), 6.54 (d, 1 H, J = 8.8 Hz, H-1), 5.20 (bt, 1 H, J = 3.0 Hz, H-4), 4.74 (ddd, 1 H, J = 13.0, 8.8, 4.6 Hz, H-2), 4.25 (m, 3 H, H-5, 2 x H-6), 2.88 (ddd, 1 H, J = 15.5, 13.0, 3.0 Hz, H-3a), 2.23 (ddd, 1 H, J = 15.5, 4.6, 3.0 Hz, H-3e), 2.20 and 2.10 (2 S, 6 H and 2 x OAc). ^{13}C: δ = 170.41, 170.25, 167.69, 160.71, 134.44, 124.36, 131.43, 123.37, 97.81, 95.55, 90.36, 75.55, 75.13, 66.46, 66.28, 65.76, 62.38, 61.89, 60.37, 46.44, 46.03, 35.16, 33.67, 30.74, 30.41, 21.01, and 20.68, Anal. Calcd for $C_{20}H_{19}Cl_3N_2O_8$: C, 46.04; H, 3.67; N, 5.37. Found: C, 45.98; H, 3.72; N, 5.30.

Octyl 4,6-O-benzylidene-β-D-galactopyranoside (**40**).

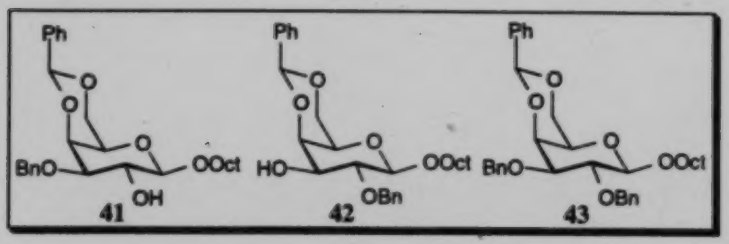


40

To a solution of **39** (42.5 g, 145 mmol) and benzaldehyde dimethyl acetal (44 ml, 291 mmol) in dried MeCN (1400 mL), *p*-TsOH (catalytic amount) was added with vigorous stirring at rt. With the reaction was going on, white solid was formed. After overnight, TLC showed the absence of the starting material (R_f = 0.54, CH_2Cl_2-MeOH, 15:1). The reaction mixture was neutralized with some Et_3N and then concentrated. The white solid was recrystalized from ethanol and the obtained white crystal was washed with cold ethanol (3 x 100 mL) to give **40** (47.4 g, 86%). NMR ($CDCl_3$): ^1H: δ = 7.38-7.30 (m, 5 H, aromatic), 5.55 (s, 1 H, O_2CHPh), 4.28 (d, 1 H, J = 7.5 Hz, H-1), 4.35 (dd, 1 H, J = 1.5, 12.5 Hz, H-6a), 4.20 (dd, 1 H, J = 3.5, 0.8 Hz, H-4), 4.08 (dd, 1 H, J = 1.5, 12.5 Hz, H-6b), 4.00 (m, 1 H, H-3), 3.94 and 3.50 (m, 2 H, $OCH_2(CH_2)_6CH_3$), 4.80-3.65 (m, 2 H, H-2 and H-3), 3.48 (bd, 1 H, J = 1.5 Hz, H-5), 2.59 (d, 1 H, J = 8.5 Hz, *OH*), 2.52 (bd, 1 H, *OH*), 1.68 (m, 2 H,

$OCH_2CH_2(CH_2)_5CH_3$), 1.40-1.20 (m, 10 H, $O(CH_2)_2(CH_2)_5CH_3$), and 0.88 (t, 3 H, $O(CH_2)_7CH_3$).

Octyl 3-O-benzyl-4,6-O-benzylidene-β-D-galactopyranoside (**41**), *Octyl 2-O-benzyl-4,6-O-benzylidene-β-D-galactopyranoside* (**42**), *Octyl 2,3-di-O-benzyl-4,6-O-benzylidene-β-D-galactopyranoside* (**43**).



To a solution of compound **40** (15 g, 39.5 mmol) and Bu_4NBr (6.45 g, 20 mmol) in CH_2Cl_2 (500 mL), BnBr (7.04 mL, 59.2 mmol) and aq NaOH (10%, 40 mL) were added at rt. The mixture was stirred vigorously overnight. The reaction solution was diluted with CH_2Cl_2 (300 mL) and washed with water. The organic layer was dried with $MgSO_4$ and evaporated. Column chromatography of the residue (toluene-EtOAc, 8:1) afforded products **41** (7.1 g, 51%), **42** (2.8 g, 28%), and **43** (1.4 g, 6%).

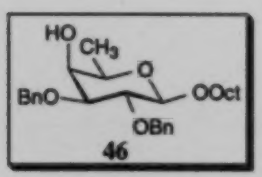
^1H NMR ($CDCl_3$) of **41**: δ = 7.60-7.30 (m, 10 H, aromatics), 5.45 (s, 1 H, O_2CHPh), 4.65 (s, 2 H, OCH_2Ph), 4.28 (d, 1 H, J = 7.1 Hz, H-1), 4.31 (dd, 1 H, J = 1.3, 12.3 Hz, H-6a), 4.13 (d, 1 H, J = 3.4 Hz, H-4), 4.04 (dd, 1 H, J = 1.7, 12.3 Hz, H-6b), 4.00 (m, 1 H, H-3), 3.94 and 3.48 (m, 2 H, $OCH_2(CH_2)_6CH_3$), 4.48 (m, 1 H, H-2), 3.36 (bs, 1 H, H-5), 2.4 (bd, 1 H, *OH*), 1.65 (m, 2 H, $OCH_2CH_2(CH_2)_5CH_3$), 1.25 (bs, 10 H, $O(CH_2)_2(CH_2)_5CH_3$), and 0.88 (t, 3 H, $O(CH_2)_7CH_3$). Selected ^1H NMR data for acetylated **41**: δ = 5.63 (dd, 1 H, J = 8.0 and 10.0 Hz, H-2).

^1H NMR ($CDCl_3$) of **42**: ^1H: δ = 7.60-7.40 (m, 10 H, aromatics), 5.55 (s, 1 H, O_2CHPh), 5.00 and 4.72 (2 d, 2 H, J = 11.2 Hz, OCH_2Ph), 4.39 (d, 1 H, J = 7.6 Hz, H-

1), 4.33 (dd, 1 H, J = 1.2, 12.5 Hz, H-6a), 4.22 (dd, 1 H, J = 3.5, 0.8 Hz, H-4), 4.07 (dd, 1 H, J = 1.8, 12.5 Hz, H-6b), 4.02 and 3.52 (m, 2 H, $OCH_2(CH_2)_6CH_3$), 3.74 (m, 1 H, H-3), 3.63 (dd, 1 H, J = 7.6, 9.5 Hz, H-2), 3.44 (bs, 1 H, H-5), 2.5 (bd, 1 H, *OH*), 1.65 (m, 2 H, $OCH_2CH_2(CH_2)_5CH_3$), 1.25 (bs, 10 H, $O(CH_2)_2(CH_2)_5CH_3$), and 0.88 (t, 3 H, $O(CH_2)_7CH_3$). Selected ^{1}H NMR data for acetylated **42**: δ = 4.92 (dd, 1 H, J = 9.5, 3.5 Hz, H-3).

^{1}H NMR ($CDCl_3$) of **43**: ^{1}H: δ = 7.60-7.20 (m, 15 H, aromatics), 5.50 (s, 1 H, O_2CHPh), 5.00 and 4.70 (m, 4 H, 2 x OCH_2Ph), 4.37 (d, 1 H, J = 8.0 Hz, H-1), 4.30 (dd, 1 H, J = 1.8, 12.5 Hz, H-6a), 4.21 (dd, 1 H, J = 3.8 and 0.8 Hz, H-4), 4.01 (dd, 1 H, J = 1.8 and 12.5 Hz, H-6b), 3.98 and 3.49 (m, 2 H, $OCH_2(CH_2)_6CH_3$), 3.85 (dd, 1 H, J = 8.0, 9.5 Hz, H-2), 3.56 (dd, 1 H, J = 3.8, 9.5 Hz, H-3), 3.31 (bs, 1 H, H-5), 1.65 (m, 2 H, $OCH_2CH_2(CH_2)_5CH_3$), 1.25 (bs, 10 H, $O(CH_2)_2(CH_2)_5CH_3$), and 0.88 (t, 3 H, $O(CH_2)_7CH_3$).

Octyl 2,3-di-O-benzyl-6-deoxy-β-D-galactopyranoside (**46**).

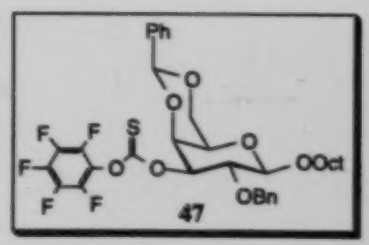


46

p-TsCl (2.059 g, 10.8 mmol) was added with stirring to a solution of compound **44** (1.2743 g, 2.7 mmol) in dry pyridine and CH_2Cl_2 (1:1, 30 mL) at -30 °C. The reaction solution was warmed to -5 °C and continued stirring for 2 h. The reaction mixture was poured into satd $NaHCO_3$ and partitioned with CH_2Cl_2. The organic layer was washed with water, dried with Na_2SO_4, concentrated and co-evaporated with toluene. To a solution of the resulting residue in dry MeCN (30 mL), Bu_4NI (7.0 g, 20 mmol) was added at rt. The mixture was refluxed overnight under Ar and then concentrated. The residue was chromatographed on a silica gel column (hexane-EtOAc, 4:1) to give crude compound **45**. To a solution of crude **45** (300 mg, about 0.4 mmol)

and Bu_3SnH (0.81 mL, 5 mmol) in dry toluene (8 mL), AIBN (catalytic amount) was added at rt. The mixture was refluxed overnight at 120 °C (oil bath temperature) and then concentrated. Finally, the resulting residue was purified by chromatograghy (hexane-EtOAc, 4:1) to yield compound **46** (218 mg, 18% from **44**; R_f = 0.55, hexane EtOAc, 3:1; $[\alpha]_D$ = - 0.8°, c = 0.7, in $CHCl_3$). NMR ($CDCl_3$): ^1H: δ = 7.40-7.20 (m, 10 H, aromatics), 4.93 and 4.72 (2 d, 2 H, J = 11.0 Hz, OCH_2Ph), 4.73 (s, 2 H, OCH_2Ph), 4.32 (d, 1 H, J = 7.8 Hz, H-1), 3.94 (dt, 1 H, J = 9.5, 6.5 Hz, O$CH(CH_2)_6CH_3$), 3.74 (bt, 1 H, H-4), 3.59 (dd, 1 H, J = 7.8, 10.0 Hz, H-2), 3.49 (m, 3 H, H-3, H-5 and O$CH(CH_2)_6CH_3$), 2.35 (bd, 1 H, *OH*), 1.65 (m, 2 H, $OCH_2CH_2(CH_2)_5CH_3$), 1.36 (d, 3 H, 3 x H-6), 1.25 (bs, 10 H, $O(CH_2)_2(CH_2)_5CH_3$), and 0.88 (t, 3 H, $O(CH_2)_7CH_3$). ^{13}C: δ = 138.78, 138.07, 128.49, 128.31, 128.13, 127.88, 127.86, 127.61, 103.62, 80.96, 78.90, 75.14, 72.45, 69.93, 69.45, 31.87, 29.82, 29.45, 29.30, 26.23, 22.70, 16.39, and 13.13. Anal. Calcd for $C_{28}H_{40}O_5$: C, 73.65; H, 8.83. Found: C, 73.55; H, 8.88.

Octyl 2-O-benzyl-4,6-O-benzylidene-3-O-pentafluorophenoxythionocarbonyl-β-D-galactopyranoside (**47**).

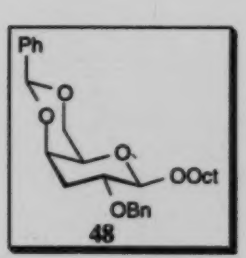


47

To a solution of compound **42** (1.070 g, 2.28 mmol) in dry CH_2Cl_2 (70 mL) containing DMAP (1.390 g, 11.4 mmol), pentafluorophenyl chlorothionoformate (1.110 mL, 6.84 mmol) was added at 0 °C. The reaction mixture was warmed to rt. After 5 h, the solution was diluted with CH_2Cl_2 and sequentially washed with 0.5% HCl, water, satd $NaHCO_3$ and water, dried with Na_2SO_4, and then concentrated. The residue was passed through a silica gel column (hexane-EtOAc, 4:1) to give compound **47** (1.586 g,

98%; R_f = 0.76, hexane-EtOAc, 2:1; $[\alpha]_D$ = + 67°, *c* = 0.6, in $CHCl_3$). NMR ($CDCl_3$): ^1H: δ = 7.60-7.20 (2 m, 10 H, aromatics), 5.60 (s, 1 H, O_2CHPh), 5.38 (dd, 1 H, J = 10.0, 3.8 Hz, H-3), 4.93 and 4.70 (2 d, 2 H, J = 11.0 Hz, OCH_2Ph), 4.64 (dd, 1 H, J = 3.8, 0.8 Hz, H-4), 4.52 (d, 1 H, J = 7.8 Hz, H-1), 4.37 and 4.10 (2 dd, 2 H, J = 12.5, 1.5 Hz, 2 x H-6), 4.04 (dd, 1 H, J = 7.8, 10.0 Hz, H-2), 4.02 and 3.52 (2 m, 2 H, $OCH_2(CH_2)_6CH_3$), 3.52 (bt, 1 H, H-5), 1.70 (m, 2 H, $OCH_2CH_2(CH_2)_5CH_3$), 1.35 (bs, 10 H, $O(CH_2)_2(CH_2)_5CH_3$), and 0.88 (t, 3 H, $O(CH_2)_7CH_3$). ^{13}C: δ = 191.55, 138.12, 137.47, 129.06, 128.35, 128.19, 127.91, 127.79, 126.29, 103.44, 101.00, 84.92, 76.17, 75.23, 72.43, 70.37, 69.04, 65.92, 31.88, 29.71, 29.47, 29.31, 26.19, 22.71, and 14.13. Anal. Calcd for $C_{35}H_{57}F_5O_7S$: C, 60.34; H, 5.53. Found: C, 60.52; H, 5.48.

Octyl 2-O-benzyl-4,6-O-benzylidene-3-deoxy-β-D-xylopyranoside (**48**).

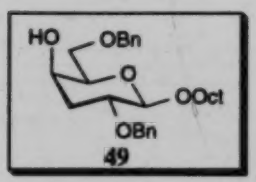


48

A solution of compound **47** (850 mg, 1.22 mmol), Bu_3SnH (1.718 mL, 6.4 mmol) and AIBN (105 mg, 0.64 mmol) in dry toluene (80 mL) was refluxed at 125 °C (oil bath temperature) under Ar for 2 h and then concentrated to dryness. The resulting residue was purified by column chromatography (hexane-EtOAc, 5:1) to give compound **48** (459 mg, 83%; R_f = 0.56, hexane-EtOAc, 3:1; $[\alpha]_D$ = - 21°, *c* = 0.7, in $CHCl_3$). NMR ($CDCl_3$): ^1H: δ = 7.55-7.20 (m, 10 H, aromatics), 5.50 (s, 1 H, O_2CHPh), 4.93 and 4.63 (2 d, 2 H, J = 11.5 Hz, OCH_2Ph), 4.42 (d, 1 H, J = 7.9 Hz, H-1), 4.32 (dd, 1 H, J = 12.5, 1.0 Hz, H-6a), 4.10-3.98 (m, 3 H, H-6b, H-4 and $OCH(CH_2)_6CH_3$), 3.74 (ddd, 1 H, J = 11.5, 7.9, 5.2 Hz, H-2), 3.52 (dt, 1 H, J = 9.3, 7.0 Hz, $OCH(CH_2)_6CH_3$), 3.46 (bd, 1 H, J = 1.0 Hz, H-5), 2.41 (ddd, 1 H, J = 14.0, 5.2, 2.9 Hz, H-3e), 1.75 (ddd, 1 H, J = 14.0, 11.5, 3.8 Hz, H-3a), 1.65,

1.30 and 0.9 (3 m, 15 H, $OCH_2(CH_2)_6CH_3$). ^{13}C: δ = 138.99, 138.10, 129.01, 128.31, 128.22, 127.71, 127.48, 126.44, 125.97, 105.62, 101.35, 73.43, 73.39, 72.90, 69.59, 69.51, 68.89, 35.26, 31.87, 29.78, 29.50, 29.32, 26.24, 22.70, 17.56, and 14.13. Anal. Calcd for $C_{28}H_{38}O_5$: C, 73.98; H, 8.43. Found: C, 73.53; H, 8.33.

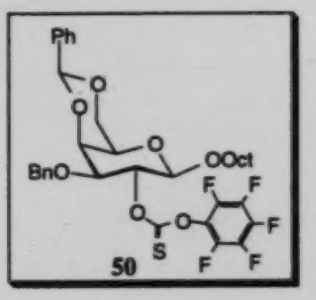
Octyl 2,6-di-O-benzyl-3-deoxy-β-D-xylopyranoside (**49**).



49

A solution of compound **48** (426 mg, 0.94 mmol), $NaCNBH_3$ (658 mg, 9.4 mmol), 3 Å molecular sieves (powder, 1.0 g), and methyl orange (little) in dry THF (20 mL) was stirred at rt for 1 h and then cooled to 0 °C. To the resulting mixture, HCl in Et_2O was added until the solution color became red. After 1 h, the mixture was diluted with CH_2Cl_2 and filtered through a Celite pad. The Celite pad was thoroughly washed with CH_2Cl_2 and the combined organic solution was sequentially washed with water, satd $NaHCO_3$ and water, dried with Na_2SO_4 and then concentrated. The residue was applied to a silica gel column (toluene-EtOAc, 5:1) to yield compound **49** (220 mg, 51%; R_f = 0.57, toluene-EtOAc, 3:1; $[\alpha]_D$ = - 17°, *c* = 0.5, in $CHCl_3$). NMR ($CDCl_3$): ^1H: δ = 7.30 (m, 10 H, aromatics), 4.85, 4.63, 4.59, and 4.54 (4 d, 4 H J = 12.0 Hz, 2 x OCH_2Ph), 4.38 (d, 1 H, J = 7.8 Hz, H-1), 3.96, 3.70, 3.62 and 3.52 (4 m, 7 H, H-2, H-4, H-5, 2 x H-6 and $OCH_2(CH_2)_6CH_3$), 2.65 (d, 1 H, J = 6.0 Hz, *OH*), 2.29 (ddd, 1 H, J = 14.0, 5.5, 3.0 Hz, H-3e), 1.60 (ddd, 1 H, J = 14.0, 11.2, 2.9 Hz, H-3a), 1.68, 1.35 and 0.88 (3 bm, 15 H, $OCH_2(CH_2)_6CH_3$). ^{13}C: δ = 138.77, 137.77, 128.49, 128.33, 127.85, 127.78, 127.52, 105.83, 75.99, 73.74, 73.16, 72.99, 70.01, 69.68, 67.28, 36.79, 32.70, 31.84, 29.80, 29.44, 29.29, 26.20, 22.68, and 14.11. Anal. Calcd for $C_{28}H_{40}O_5$: C, 73.65; H, 8.83. Found: C, 73.60; H, 8.90.

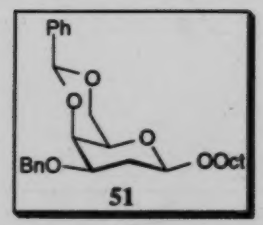
Octyl 3-O-benzyl-4,6-O-benzylidene-2-O-pentafluorophenoxythionocarbonyl-β-D-galactopyranoside (**50**).



50

To a solution of compound **41** (246 mg, 0.52 mmol) in dry CH_2Cl_2 (20 mL) containing DMAP (320 mg, 2.62 mmol), pentafluorophenyl chlorothionoformate (0.253 mL, 1.57 mmol) was added at 0 °C. The reaction mixture was warmed to rt. After 5 h, the solution was diluted with CH_2Cl_2 and washed with 0.5% HCl, water, satd $NaHCO_3$ and water, dried with Na_2SO_4, and then concentrated. The resulting residue was passed through a silica gel column (hexane-EtOAc, 2:1) to afford compound **50** (298.5 mg, 80%; R_f= 0.36, hexane-EtOAc, 2:1; $[\alpha]_D$ = - 6°, *c* = 0.4, in $CHCl_3$). NMR ($CDCl_3$): ^{1}H: δ = 7.65-7.25 (2 m, 10 H, aromatics), 5.82 (dd, 1 H, J = 10.0, 8.0 Hz, H-2), 5.54 (s, 1 H, O_2CHPh), 4.76 and 4.68 (2 d, 2 H, J = 12.5 Hz, OCH_2Ph), 4.57 (d, 1 H, J = 8.0 Hz, H-1), 4.34 (dd, 1 H, J = 12.5, 1.5 Hz, H-6a), 4.06 (dd, 1 H, J = 12.5, 1.8 Hz, 2 H-6), 4.24 (bd, 1 H, J = 3.5 Hz, H-4), 3.95 (dt, 1 H, J = 9.5, 6.3 Hz, O$CH(CH_2)_6CH_3$), 3.77 (dd, 1 H, J = 10.0, 8.0 Hz, H-3), 3.47 (dt, 1 H, J = 9.5, 7.0 Hz, O$CH(CH_2)_6CH_3$), 3.40 (bd, 1 H, J = 1.0 Hz, H-5), 1.60 (m, 2 H, $OCH_2CH_2(CH_2)_5CH_3$), 1.30 (bs, 10 H, $O(CH_2)_2(CH_2)_5CH_3$), and 0.88 (t, 3 H, $O(CH_2)_7CH_3$). ^{13}C: δ = 191.24, 137.55, 129.05, 128.49, 128.20, 127.98, 127.72, 126.48, 101.38, 100.83, 82.36, 76.95, 73.37, 71.29, 70.10, 69.06, 66.67, 33.09, 31.86, 29.48, 29.42, 29.16, 25.85, 22.67, and 14.08. Anal. Calcd for $C_{35}H_{57}F_5O_7S$: C, 60.34; H, 5.53. Found: C, 60.78; H, 5.48.

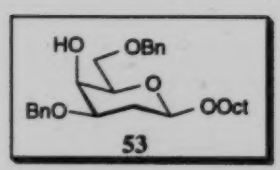
Octyl 3-O-benzyl-4,6-O-benzylidene-2-deoxy-β-D-lyxopyranoside (**51**).



51

A solution of compound **50** (1.3360 g, 1.92 mmol), Bu_3SnH (2.58 mL, 9.59 mmol) and AIBN (158 mg, 0.96 mmol) in dry toluene (115 mL) was refluxed at 125 °C (oil bath temperature) under Ar for 5 h and then was concentrated to dryness. The resulting residue was purified by column chromatography (hexane-EtOAc, 4:1) to give compound **51** (820 mg, 94%; R_f = 0.38, hexane-EtOAc, 3:1; $[\alpha]_D$ = + 8°, *c* = 0.3, in $CHCl_3$). NMR ($CDCl_3$): ^1H: δ = 7.60-7.25 (m, 10 H, aromatics), 5.55 (s, 1 H, O_2CHPh), 4.65 (s, 2 H, OCH_2Ph), 4.45 (dd, 1 H, J = 9.5, 2.0 Hz, H-1), 4.32 (dd, 1 H, J = 12.0, 1.5 Hz, H-6a), 4.10 (d, 1 H, J = 3.2 Hz, H-4), 4.07 (dd, 1 H, J = 12.0, 1.8 Hz, H-6b), 3.95 (dt, 1 H, J = 9.5, 6.8 Hz, $OCH(CH_2)_6CH_3$), 3.60 (ddd, 1 H, J = 12.0, 5.0, 3.2 Hz, H-3), 3.43 (dt, 1 H, J = 9.5, 7.0 Hz, $OCH(CH_2)_6CH_3$), 3.26 (bd, 1 H, J = 1.0 Hz, H-5), 2.16 (ddd, 1 H, J = 12.0, 9.5, 12.0 Hz, H-2a), 2.04 (ddd, 1 H, J = 12.0, 5.0, 2.0 Hz, H-2e), 1.62 , 1.30 and 0.9 (3 m, 15 H, $O(CH_2)_7CH_3$). ^{13}C: δ = 138.19, 138.10, 128.82, 128.47, 128.07, 127.75, 127.72, 126.58, 101.09, 100.37, 74.45, 72.24, 69.58, 69.80, 69.16, 66.88, 32.27, 31.84, 29.61, 29.45, 26.07, 22.67, and 14.11. Anal. Calcd for $C_{28}H_{38}O_5$:C, 73.98; H, 8.43. Found: C, 73.68; H, 8.50.

Octyl 3,6-di-O-benzyl-2-deoxy-β-D-lyxopyranoside (**53**).

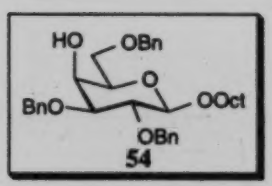


53

A mixture of compound **51** (137 mg, 0.30 mmol), borane trimethylamine (44 mg, 0.60 mmol) and 4 Å molecular sieves (1.0 g) in THF (9 mL) was stirred at rt for 1 h. To the resulting mixture, $AlCl_3$ (80 mg, 0.60 mmol) was added with stirring. After 4 h, the mixture was filtered and the filtrate was diluted with CH_2Cl_2, washed with satd $NaHCO_3$, dried with $NaSO_4$ and concentrated. The residue was subjected to silica gel column chromatography (toluene-EtOAc, 6:1) to give product **53** (19 mg, 14%; 52% of **51**

recovered; R_f = 0.62, toluene-EtOAc, 3:1; $[\alpha]_D$ = - 11°, *c* = 0.7, in $CHCl_3$). NMR ($CDCl_3$): ^1H: δ = 7.45-7.22 (m, 10 H, aromatics), 4.64 and 4.59 (2 d, 2 H, J = 12.5 Hz, O*CH*$_2$Ph), 4.61 (s, 2 H, O*CH*$_2$Ph), 4.40 (dd, 1 H, J = 9.5, 2.3 Hz, H-1), 3.98 (bt, 1 H, H-4), 3.90 (dt, 1 H, J = 9.4, 6.5 Hz, O*CH*$(CH_2)_6CH_3$), 3.83 and 3.73 (2 dd, 2 H, J = 10.0, 5.8 Hz, 2 x H-6), 3.53 (ddd, 1 H, J = 12.5, 5.0, 3.0 Hz, H-3), 3.50 (bt, 1 H, J = 5.8 Hz, H-5), 3.43 (dt, 1 H, J = 9.4, 7.0 Hz, O*CH*$(CH_2)_6CH_3$), 2.31 (bd, 1 H, J = 3.0 Hz, *OH*), 2.04 (ddd, 1 H, J = 12.5, 5.0, 2.0 Hz, H-2e), 1.86 (dt, 1 H, J = 9.5, 12.5 Hz, H-2a), 1.58 (m, 2 H, $OCH_2CH_2(CH_2)_5CH_3$), 1.27 (bs, 10 H, $O(CH_2)_2(CH_2)_5CH_3$), and 0.86 (t, 3 H, $O(CH_2)_7CH_3$). ^{13}C: δ = 138.20, 137.83, 128.57, 128.45, 127.93, 127.80, 127.73, 100.19, 75.15, 73.88, 69.93, 69.66, 69.43, 65.13, 32.29, 31.85, 29.67, 29.43, 29.27, 26.09, 22.69, 14.13, and 12.66. Anal. Calcd for $C_{28}H_{40}O_5$: C, 73.65; H, 8.83. Found: C, 73.48; H, 8.85.

Octyl 2,3,6-tri-O-benzyl-β-D-galactopyranoside (**54**).

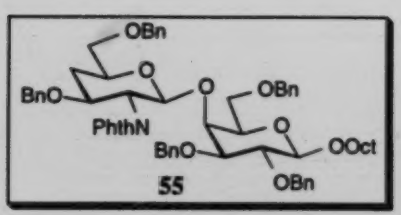


54

A solution of compound **43** (2 g, 3.57 mmol), $NaCNBH_3$ (2.25 g, 35.7 mmol), 3 Å molecular sieves (powder, 14 g) and methyl orange (little) in THF (70 mL) was stirred at rt for 1 h and then HCl in Et_2O was added in dropwise until the solution became red color. After 5 h, the mixture was diluted with CH_2Cl_2 and filtered through Celite. The filtrate was sequentially washed with water, satd $NaHCO_3$ and water, dried with Na_2SO_4 and then concentrated. The resulting residue was applied to a silica gel column (toluene-EtOAc, 6:1 to 3:1) to yield compound **54** (1.68 g, 84 %; R_f = 0.60, toluene-EtOAc, 3:1; $[\alpha]_D$ = - 2°, *c* = 1.7, in $CHCl_3$) and Octyl 2,3,4-tri-*O*-benzyl-β-D-galactopyranoside (0.25 g, 12%). NMR ($CDCl_3$) of **54**: ^1H: δ = 7.42-7.23 (m, 15 H, aromatics), 4.92 and 4.72 (2 d, 2 H, J = 11.0 Hz, O*CH*$_2$Ph), 4.72 and 4.59 (2 s, 4 H, 2 x

O*CH*$_2$Ph), 4.34 (d, 1 H, J = 7.8 Hz, H-1), 4.02 (bt, 1 H, J = 2.0 Hz, H-4), 3.95 (dt, 1 H, J = 9.4, 6.5 Hz, O*CH*$(CH_2)_6CH_3$), 3.81 (dd, 1 H, J = 10.0, 5.5 Hz, H-6a), 3.72 (dd, 1 H, J = 5.5, 10.0 Hz, H-6b), 3.64 (dd, 1 H, J = 7.8, 9.2 Hz, H-2), 3.56 (t, 1 H, J = 5.5 Hz, H-5), 3.54-3.46 (m, 2 H, H-3 and O*CH*$(CH_2)_6CH_3$), 1.68, 1.35 and 0.88 (3 bm, 15 H, OCH_2*$(CH_2)_6CH_3$*). Anal. Calcd for $C_{28}H_{40}O_5$: C, 74.70; H, 8.24. Found: C, 73.50; H, 8.36.

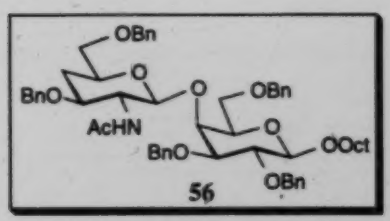
Octyl 4-O-(3,6-di-O-benzyl-2,4-dideoxy-2-phthalimido-β-D-xylopyranosyl)-2,3,6-tri-O-benzyl-β-D-galactopyranoside (**55**).



A mixture of **14** (145 mg, 0.235 mmol), **54** (190 mg, 0.336 mmol) and AW-300 molecular sieves (powder, 200 mg) in dry MeCN (3 mL) and CH_2Cl_2 (1 mL) was stirred at rt about 30 min, and then cooled to - 50 °C. To the cooled mixture, TMSOTf (2.3 µL, 12 µmol) in dry MeCN (0.1 mL) was added. After 10 min, TLC indicated the absence of **14**. The mixture was neutralized with Et_3N, diluted with CH_2Cl_2 (20 mL) and filtered. The filtrate was concentrated. The residue was eluted from a silica gel column (toluene-EtOAc, 16:1) to give disaccharide **55** (170 mg, 71%; *Rf* = 0.71, hexane-EtOAc, 2:1; $[\alpha]_D$ = - 7°, *c* = 0.4, in CCl_4). NMR ($CDCl_3$): ^{1}H: δ = 7.85-6.95 (m, 29 H, aromatics), 5.17 (d, 1 H, J = 8.3 Hz, H-1'), 4.61 (s, 1 H, J = 12.5 Hz, O*CH*Ph), 4.56 (ddd, 1 H, J = 11.0, 11.0, 5.0 Hz, H-3'), 4.48 (s, 4 H, 2 x O*CH*$_2$Ph), 4.38 (d, 1 H, J = 10.5 Hz, O*CH*Ph), 4.37 and 4.36 (2 d, 2 H, J = 12.5 Hz, O*CH*$_2$Ph), 4.19 (dd, 1 H, J = 11.0, 8.3 Hz, H-2'), 4.17 (d, 1 H, J = 12.5 Hz, O*CH*Ph), 4.11 (bd, 1 H, J = 7.0 Hz, H-1), 3.83 (dt, 1 H, J = 9.5, 6.5 Hz, O*CH*$(CH_2)_6CH_3$), 3.78 (dd, 1 H, J = 10.0, 5.5 Hz, H-6a), 3.74 (dtd, 1 H, J = 12.0, 5.5, 1.5 Hz, H-5'), 3.63 (dd, 1 H, J = 10.0, 6.0 Hz, H-6'a), 3.62 (bd, 1 H, H-4), 3.56 (dd, 1

H, J = 10.0, 5.0 Hz, H-6'b), 3.55 (d, 1 H, J = 10.5 Hz, O*CH*Ph), 3.45 (dd, 1 H, J = 10.0, 5.5 Hz, H-6b), 3.37 (dt, 1 H, J = 9.5, 7.0 Hz, O*CH*$(CH_2)_6CH_3$), 3.30 (bt, 1 H, J = 5.5 Hz, H-5), 3.10 (m, 2 H, H-2 and H-3), 2.65 (ddd, 1 H, J = 13.0, 5.0, 1.5 Hz, H-4e'), 1.60 (bm, 3 H, H-4a', $OCH_2CH_2(CH_2)_5CH_3$), 1.25 (bs, 10 H, $O(CH_2)_2(CH_2)_5CH_3$), and 0.85 (t, 3 H, $O(CH_2)_7CH_3$). ^{13}C: δ = 168.61, 167.93, 138.62, 138.58, 138.46, 138.29, 138.09, 133.38, 132.76, 132.09, 129.06, 128.48, 128.33, 128.24, 128.14, 127.95, 127.75, 127.62, 127.60, 127.51, 127.41, 127.36, 125.33, 123.16, 122.73, 103.28, 100.08, 79.91, 79.55, 76.19, 74.96, 73.52, 73.37, 72.64, 72.50, 70.91, 70.61, 70.01, 69.64, 56.98, 34.39, 31.88, 29.73, 29.48, 29.29, 26.18, 22.70, 21.47, and 14.14. Anal. Calcd for $C_{63}H_{71}NO_{11}$: C, 74.31; H, 7.03; N, 1.38. Found: C, 74.05; H, 7.30; N, 1.22.

Octyl 4-O-(2-acetamido-2,4-dideoxy-3,6-di-O-benzyl-β-D-xylopyranosyl)-2,3,6-tri-O-benzyl-β-D-galactopyranoside (**56**).

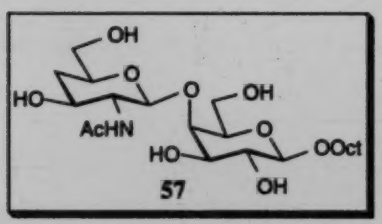


56

A solution of compound **55** (149 mg, 0.146 mmol) in ethanol (98%, 15 mL) and $NH_2NH_2{\cdot}H_2O$ (1.5 mL) was refluxed at 90 °C (oil bath temperature) for approximately 1 h at which point TLC indicated the reaction had gone to completion (R_f = 0.15, CH_2Cl_2-MeOH, 10:1). The solution was concentrated and co-evaporated three times with ethanol to remove the excess hydrazine followed by co-evaporation with toluene twice. A solution of the residue in pyridine-Ac_2O (1:1, 10 mL) was stirred overnight at rt, concentrated and co-evaporated with toluene. The resulting residue was chromatographed on a silica gel column (hexane-EtOAc, 2:1) to give product **56** (131 mg, 96%; R_f = 0.73, hexane-EtOAc, 1:1; $[\alpha]_D$ = + 13°, *c* = 0.7, in $CHCl_3$). NMR ($CDCl_3$): ^1H: δ = 7.40-7.20 (m, 25 H, aromatics), 5.67 (d, 1 H, J = 7.5 Hz, *NH*), 4.99 and 4.60 (2 d, 2 H, J = 11.0 Hz,

O*CH*$_2$Ph), 4.77 and 4.63 (2 d, 2 H, J = 10.5 Hz, O*CH*$_2$Ph), 4.55 and 4.40 (2 d, 2 H, J = 12.5 Hz, O*CH*$_2$Ph), 4.52 and 4.46 (2 s, 4 H, 2 x O*CH*$_2$Ph), 4.53 (d, 1 H, J = 7.5 Hz, H-1'), 4.32 (d, 1 H, J = 7.4 Hz, H-1), 3.95 (bs, 1 H, H-4), 3.94 (dt, 1 H, J = 9.5, 6.5 Hz, O*CH*(CH$_2$)$_6$CH$_3$), 3.81 (dd, 1 H, J = 10.0, 5.5 Hz, H-6a), 3.66 (dd, 1 H, J = 10.0, 6.0 Hz, H-6b), 3.62-3.45 (m, 8 H, H-2, H-3, H-5, H-2', H-3', 2 x H-6' and O*CH*(CH$_2$)$_6$CH$_3$), 3.40 (m, 1 H, H-5'), 2.10 (bdd, 1 H, H-4e'), 1.71 (s, 3 H, NAc), 1.62 (bm. 2 H, OCH$_2$*CH*$_2$(CH$_2$)$_5$CH$_3$), 1.40 (bm, 1 H, H-4a'), 1.28 (bs, 10 H, O(CH$_2$)$_2$*(CH*$_2$*)*$_5$CH$_3$), and 0.87 (t, 3 H, O(CH$_2$)$_7$*CH*$_3$). ^{13}C: δ = 169.94, 138.76, 138.71, 138.53, 138.04, 127.72, 128.67, 128.46, 128.42, 128.38, 128.36, 128.27, 127.83, 127.75, 127.62, 127.56, 127.42, 103.74, 102.47, 81.45, 79.88, 77.41, 75.16, 75.12, 74.05, 73.70, 73.51, 72.67, 71.28, 70.95, 69.95, 69.89, 56.76, 33.92, 31.84, 29.73, 29.43, 29.25, 26.19, 23.52, 22.66, and 14.09. Anal. Calcd for $C_{57}H_{71}NO_{10}$: C, 73.60; H, 7.69; N, 1.51. Found: C, 73.45; H, 7.75; N, 1.49.

Octyl 4-O-(2-acetamido-2,4-dideoxy-β-D-galactopyranosyl)-β-D-galactopyranoside (**57**).

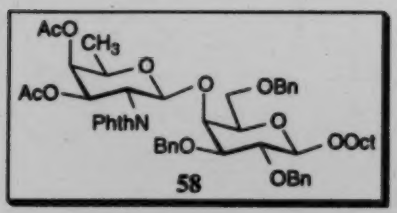


57

Compound **56** (131 mg, 0.141 mmol) was hydrogenolyzed over Pearlman's catalyst ($Pd(OH)_2$/C, 20%, 30 mg) in ethanol (98%, 15 mL) for 14 h. The mixture was filtered through Celite and the filtrate was concentrated. The resulting residue was purified by column chromatography (5:1, CH_2Cl_2-MeOH containing 1.5% of H_2O) to give compound **57** (54 mg, 80%; R_f = 0.27, 5:1, CH_2Cl_2-MeOH containing 1.5% of H_2O; $[\alpha]_D$ = - 19°, *c* = 0.5, in MeOH). Selected NMR (500 MHz, CD_3OD): ^{1}H: δ = 4.58 (d, 1 H, J = 8.4 Hz, H-1'), 4.18 (d, 1 H, J = 7.8 Hz, H-1). 1.92 (ddd, 1 H, H-4e'), 1.32 (m, 1 H, H-4a'), and 2.03 (s, 3 H, NAc). ^{13}C: δ = 105.45 (C-1, J_{C1H1} = 158.1 Hz,) and 104.05 (C-1', $J_{C1'H1'}$

= 161.2 Hz). FAB-MS: Calcd for $C_{22}H_{41}NO_{10}$ $[M+Na]^+$ and $[M+H]^+$: *m/z* 502 and 480. Found: *m/z* 502 and 480.

Octyl 4-O-(3,4-di-O-acetyl-2,6-dideoxy-2-phthalimido-β-D-galactopyranosyl)-2,3,6-tri-O-benzyl-β-D-galactopyranoside (**58**).

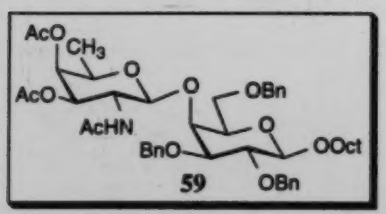


58

A solution of compound **25** (55 mg, 0.130 mmol), compound **54** (106 mg, 0.182 mmol) and AW-300 molecular sieves (powder, 100 mg) in dry MeCN (1 mL) and dry CH_2Cl_2 (0.3 mL) was stirred for about 30 min at rt under Ar and then cooled to - 50 °C. To the cooled mixture, a solution of NIS (61 mg, 0.260 mmol) and TfOH (1.2 μL, 0.013 mmol) in dry MeCN (0.1 mL) was added. After 20 min, the solution was diluted with CH_2Cl_2 (20 mL), neutralized with Et_3N (2 mL) and filtered. The filtrate was successively washed with aq $Na_2S_2O_3$ and satd $NaHCO_3$, dried with Na_2SO_4, and concentrated. The resulting residue was passed through a silica gel column (hexane-EtOAc, 4:1) to yield compound **58** (100 mg, 83%; R_f = 0.52, hexane-EtOAc, 2:1; $[\alpha]_D$ = - 14°, *c* = 0.4, in $CHCl_3$). NMR ($CDCl_3$): ^1H: δ = 7.92-7.02 (m, 19 H, aromatics), 6.11 (dd, 1 H, J = 11.5, 3.4 Hz, H-3'), 5.37 (d, 1 H, J = 3.5 Hz, H-4'), 5.32 (d, 1 H, J = 8.5 Hz, H-1'), 4.67 (dd, 1 H, J = 11.5, 8.5 Hz, H-2'), 4.60 and 4.54 (2 d, 2 H, J = 11.5 Hz, O*CH*$_2$Ph), 4.48 and 3.63 (2 d, 2 H, J = 10.5 Hz, O*CH*$_2$Ph), 4.43 and 4.22 (2 d, 2 H, J = 12.5 Hz, O*CH*$_2$Ph), 4.20 (d, 1 H, J = 7.3 Hz, H-1), 3.98 (bq, 1 H, J = 6.3 Hz, H-5'), 3.93 (dt, 1 H, J = 9.5, 6.5 Hz, O*CH*$(CH_2)_6CH_3$), 3.88 (dd, 1 H, J = 9.5, 6.5 Hz, H-6a), 3.71 (d, 1 H, J = 2.8 Hz, H-4), 3.68 (dd, 1 H, J = 9.5, 6.5 Hz, H-6b), 3.44 (dt, 1 H, J = 9.5, 7.0 Hz, O*CH*$(CH_2)_6CH_3$), 3.37 (bt, 1 H, J = 6.0 Hz, H-5), 3.22 (dd, 1 H, J = 9.5, 2.8 Hz, H-3), 3.15 (dd, 1 H, J = 9.5, 7.3 Hz, H-2), 2.25 and 1.90 (2 s, 6 H, 2 x OAc), 1.65 (bm, 2 H,

$OCH_2CH_2(CH_2)_5CH_3$), 1.30 (bs, 10 H, $O(CH_2)_2(CH_2)_5CH_3$), 1.20 (d, 3 H, J = 6.3 Hz, 3 x H-6'), and 0.90 (t, 3 H, $O(CH_2)_7CH_3$). ^{13}C: δ = 170.76, 169.91, 168.48, 167.72, 138.42, 138.38, 138.07, 133.76, 133.57, 132.53, 131.79, 128.41, 128.34, 128.25, 128.11, 127.87, 127.71, 127.58, 127.45, 123.21, 123.10, 103.34, 99.72, 79.95, 79.65, 76.58, 74.98, 73.33, 73.10, 72.73, 69.85, 69.31, 68.52, 67.97, 51.28, 31.82, 29.66, 29.42, 29.22, 26.13, 22.65, 20.73, 20.63, 16.40, and 14.08. Anal. Calcd for $C_{53}H_{63}NO_{13}$: C, 69.04; H, 6.89; N, 1.52. Found: C, 68.90; H, 7.00; N, 1.50.

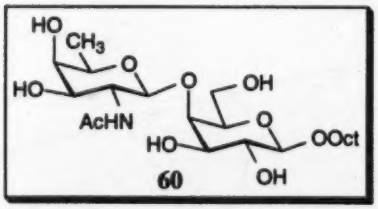
Octyl 4-O-(2-acetamido-3,4-di-O-acetyl-2,6-dideoxy-β-D-galactopyranosyl)-2,3,6-tri-O-benzyl-β-D-galactopyranoside (**59**).



A solution of compound **58** (88 mg, 0.096 mmol) in ethanol (98%, 10 mL) and hydrazine monohydrate (1.0 mL) was refluxed at 90 °C (oil bath temperature) for 1 h, with TLC indicating reaction was complete (R_f = 0.24, CH_2Cl_2-MeOH, 10:1). The solution was concentrated and co-evaporated with ethanol three times to remove the excess of hydrazine followed by co-evaporation with toluene twice. The residue was dissolved in pyridine-Ac_2O (1:1, 10 mL) and the solution was kept overnight at rt, concentrated and co-evaporated with toluene. The residue was applied to a silica gel column (hexane-EtOAc, 1:1) to give compound **59** (71 mg, 89%; R_f = 0.37, hexane-EtOAc, 1:1; $[\alpha]_D$ = + 29°, *c* = 0.3, in $CHCl_3$). NMR (CD_3OD): ^1H: δ = 7.45-7.22 (m, 15 H, aromatics), 5.70 (d, 1 H, J = 7.8 Hz, *NH*), 5.10 (bd, 1 H, J = 3.0 Hz, H-4'), 4.99 and 4,61 (2 d, 2 H, J = 10.8 Hz, OCH_2Ph), 4.81 and 4.67 (2 d, 2 H, J = 10.5 Hz, OCH_2Ph), 4.80 (dd, 1 H, J = 11.0, 3.5 Hz, H-3'), 4.60 and 4.54 (2 d, 2 H, J = 12.5 Hz, OCH_2Ph), 4.58 (d, 1 H, J = 8.5 Hz, H-1'), 4.34 (m, 1 H, virtual coupling, similar to compound 2 in reference [80], H-1), 4.12 (dt, 1 H, J = 10.8, 8.5 Hz, H-2'), 4.10 (bs, 1 H, H-4), 3 93 (dt, 1 H, J = 9.5, 6.5 Hz, $OCH(CH_2)_6CH_3$),

3.87 (dd, 1 H, J = 9.5, 6.5 Hz, H-6a), 3.68 (bq, 1 H, J = 6.3 Hz, H-5'), 3.67 (dd, 1 H, J = 9.5, 5.2 Hz, H-6b), 3.56 (d, 2 H, H-2 and H-3), 3.54 (bt 1 H, J = 6.3 Hz, H-5), 3.47 (dt, 1 H, J = 9.5, 7.0 Hz, O*CH*$(CH_2)_6CH_3$), 2.18 and 1.98 (2 s, 6 H, 2 x OAc), 1.67 (m, 2 H, $OCH_2CH_2(CH_2)_5CH_3$), 1.65 (s, 3 H, NAc), 1.30 (bm, 10 H, $O(CH_2)_2(CH_2)_5CH_3$), 1.12 (d, 3 H, J = 6.3 Hz, 3 x H-6'), and 0.88 (t, 3 H, $O(CH_2)_7CH_3$). ^{13}C: δ = 170.81, 170.56, 169.92, 138.68, 138.35, 137.44, 128.94, 128.66, 128.51, 128.45, 128.39, 128.35, 128.33, 128.32, 128.30, 128.28, 128.26, 128.24, 128.06, 127.76, 127.70, 127.65, 103.80, 102.79, 81.50, 80.01, 76.00, 75.14, 74.44, 73.49, 73.23, 72.45, 69.98, 69.40, 69.12, 50.78, 31.82, 29.71, 29.41, 26.18, 23.16, 22.65, 20.79, 20.76, 16.23, and 14.08. Anal. Calcd for $C_{47}H_{63}NO_{12}$: C, 67.96; H, 7.61; N, 1.68. Found: C, 67.90; H, 7.67; N, 1.66.

Octyl 4-O-(2-acetamido-2,6-dideoxy-β-D-galactopyranosyl)-β-D-galactopynoside (**60**).

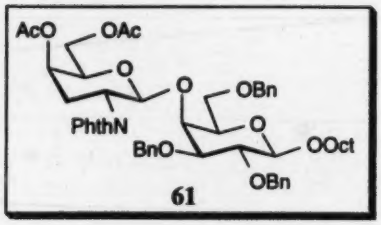


60

NaOMe (27 mg) was added to a solution of compound **59** (71 mg, 0.085 mmol) in dry MeOH (10 mL). The mixture was stirred for 1 h with completion verified by TLC (R_f = 0.61, CH_2Cl_2-MeOH, 10:1). The solution was neutralized with Dowex 50W-X8 $[H]^+$ resin, filtered, and concentrated. The crude product was hydrogenolyzed over $Pd(OH)_2/C$ (20%, 14 mg) in ethanol (98%, 10 mL) for 15 h, filtered through Celite and concentrated. The residue was purified by column chromatography (5:1, CH_2Cl_2-MeOH containing 1.5% of H_2O) to give compound **60** (30 mg, 74%; R_f = 0.33, 5:1, CH_2Cl_2-MeOH containing 1.5% of H_2O; $[\alpha]_D$ = - 15°, *c* = 0.6, in MeOH). Selected NMR (500 MHz, CD_3OD): ^1H: δ = 4.67 (d, 1 H, J = 8.6 Hz, H-1'), 4.17 (d, 1 H, J = 7.8 Hz, H-1), 2.20 (s, 3 H, NAc), and 1.28 (d, 3 H, 3 x H-6'). ^{13}C: δ = 104.40 (C-1, J_{C1H1} = 158.1 Hz) and 103.55 (C-1', $J_{C1'H1'}$ =

161.6 Hz). FAB-MS: Calcd for $C_{22}H_{41}NO_{10}$ $[M+Na]^+$ and $[M+H]^+$: *m/z* 502 and 480. Found: *m/z* 502 and 480.

Octyl 4-O-(4,6-di-O-acetyl-2,3-dideoxy-2-phthalimido-β-D-xylopyranosyl)-2,3,6-tri-O-benzyl-β-D-galactopyranoside (**61**).

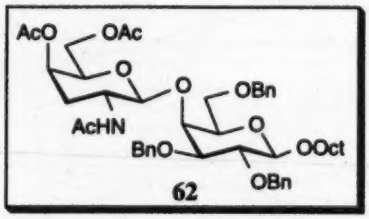


61

A mixture of compound **37** (105 mg, 0.201 mmol), compound **54** (170 mg, 0.302 mmol) and AW-300 molecular sieves (powder, 300 mg) in dry MeCN (3 mL) and CH_2Cl_2 (1 mL) was stirred at rt for about 30 min and cooled to - 50 °C. To the cooled mixture, TMSOTf (4.0 μL, 0.0201 mmol) in dry MeCN (0.1 mL) was added. After 30 min, TLC indicated the absence of donor **37**. The mixture was then neutralized with Et_3N (2 mL), diluted with CH_2Cl_2 (20 mL) and filtered. The filtrate was concentrated. The residue was eluted from a silica gel column (hexane-EtOAc, 3:1) to give disaccharide **61** (160 mg, 86%; R_f = 0.38, hexane-EtOAc 2:1; $[\alpha]_D$ = - 28°, *c* = 0.6, in $CHCl_3$). NMR ($CDCl_3$): ^1H: δ = 7.60-7.00 (m, 19 H, aromatics), 5.28 (d, 1 H, J = 8.4 Hz, H-1'), 5.13 (bt, 1 H, H-4'), 4.57 (ddd, 1 H, J = 13.5, 8.4, 5.0 Hz, H-2'), 4.54 (s, 2 H, O*CH*$_2$Ph), 4.43 and 3.60 (2 d, 2 H, J = 10.4 Hz, O*CH*$_2$Ph), 4.38 and 4.19 (2 d, 2 H, J = 12.5 Hz, O*CH*$_2$Ph), 4.18 (dd, 1 H, J = 12.5, 8.8 Hz, H-6a'), 4.03 (m, 2 H, H-5' and H-6b'), 3.88 (dt, 1 H, J = 9.5, 6.5 Hz, O*CH*$(CH_2)_6CH_3$), 3.77 (dd, 1 H, J = 10.5 5.5 Hz, H-6a), 3.67 (dd, 1 H, J = 10.5, 6.0 Hz, H-6b), 3.62 (bd, 1 H, H-4), 3.40 (dt, 1 H, J = 9.5, 7.0 Hz, O*CH*$(CH_2)_6CH_3$), 3.33 (bt, 1 H, J = 5.5 Hz, H-5), 3.18 (dd, 1 H, J = 9.5, 2.5 Hz, H-3), 3.11 (dd, 1 H, J = 9.5, 7.3 Hz, H-2), 3.02 (btd, 1 H, J = 13.5, 3.0 Hz, H-3a'), 2.15 (m, 1 H, H-3e'), 2.16 and 2.01 (2 s, 6 H, 2 x OAc), 1.60 (m, 2 H, $OCH_2CH_2(CH_2)_5CH_3$), 1.25 (bs, 10 H, $O(CH_2)_2(CH_2)_5CH_3$), and 0.87 (t, 3 H,

$O(CH_2)_7CH_3$). ^{13}C: δ = 170.49, 138.49, 138.46, 138.19, 133.70, 128.40, 128.30, 128.16, 127.92, 127.74, 127.65, 127.60, 127.49, 123.08, 103.31, 100.97, 79.93, 79.72, 76.71, 75.02, 73.54, 73.49, 73.41, 72.73, 70.01, 69.81, 66.71, 62.37, 47.32, 31.87, 29.95, 29.73, 29.48, 29.28, 26.19, 22.70, 21.14, 20.75, and 14.13. Anal. Calcd for $C_{53}H_{63}NO_{13}$: C, 69.04; H, 6.89; N, 1.52. Found: C, 58.88; H, 7.01; N, 1.51.

Octyl 4-O-(2-acetamido-4,6-di-O-acetyl-2,3-dideoxy-β-D-xylopyranosyl)-2,3,6-tri-O-benzyl-β-D-galactopyranoside (**62**).

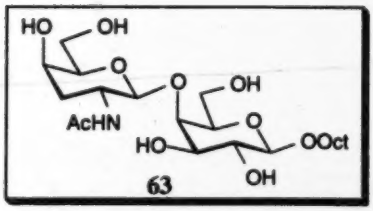


62

A solution of compound **61** (140 mg, 0.152 mmol) hydrazine monohydrate (1.0 mL) in ethanol (98%, 10 mL) was refluxed for 1 h, with TLC indicating the reaction had gone to completion (R_f = 0.25, CH_2Cl_2-MeOH, 10:1). The solution was concentrated and co-evaporated with ethanol three times to remove the excess of hydrazine followed by co-evaporation with toluene twice. The residue was dissolved in pyridine-Ac_2O (1:1, 10 mL). The solution was kept overnight at rt, concentrated and co-evaporated with toluene. The residue was applied to a silica gel column (hexane-EtOAc, 1:1) to give compound **62** (120.3 mg, 95%; R_f = 0.19, hexane-EtOAc, 1:1; [α] = - 2°, *c* 1.1, in MeOH). NMR ($CDCl_3$): ^1H: δ = 7.44-7.16 (m, 15 H, aromatics), 4.95 and 4.64, 4.76 and 4.71 (4 d, 4 H, J = 11.5 Hz, 2 x OCH_2Ph), 4.93 (bs, 1 H, H-4'), 4.68 (d, 1 H, J = 8.5 Hz, H-1'), 4.57 (s, 2 H, OCH_2Ph), 4.40 (d, 1 H, J = 7.6 Hz, H-1), 4.12 (bd, 1 H, J = 2.5 Hz, H-4), 4.11 (dd, 1 H, J = 11.5, 6.3 Hz, H-6a'), 4.02 (dd, 1 H, J = 11.6, 6.5 Hz, H-6b'), 3.91 (bt, 1 H, J = 6.0 Hz, H-5'), 3.90 (dt, 1 H, J = 9.5, 6.5 Hz, $OCH(CH_2)_6CH_3$), 3.80-3.64 (m, 5 H, H-3, H-2', H-5 and 2 x H-6), 3.54 (dt, 1 H, J = 9.5, 6.0 Hz, $OCH(CH_2)_6CH_3$), 3.50 (dd, 1 H, J = 10.0, 7.5 Hz, H-2), 2.38 (ddd, 1 H, J = 14.5, 5.0, 3.0 Hz, H-3e'), 2.07 and 1.97 (2 s, 6 H, 2 x OAc), 1.52 (s, 3 H, NAc), 1.49

(ddd, 1 H, J = 14.5, 12.0, 2.5 Hz, H-3a'), 1.60 (m, 2 H, $OCH_2CH_2(CH_2)_5CH_3$), 1.25 (bs, 10 H, $O(CH_2)_2(CH_2)_5CH_3$), and 0.88 (t, 3 H, $O(CH_2)_7CH_3$). ^{13}C: δ = 172.59, 172.10, 171.96, 140.17, 139.70, 138.90, 133.88, 130.13, 129.76, 129.52, 129.37, 129.33, 128.71, 128.61, 126.53, 105.05, 104.84, 82.89, 81.19, 76.94, 76.19, 75.63, 75.15, 74.51, 74.36, 71.32, 70.91, 67.99, 63.59, 48.85, 34.05, 32.92, 31.12, 30.84, 30.43, 30.35, 27.28, 23.66, 22.85, 20.91, 20.71, and 14.45. Anal. Calcd for $C_{47}H_{63}NO_{12}$: C 67.96, H 7.61, N 1.68. Found: C 67.60, H 7.65, N 1.67.

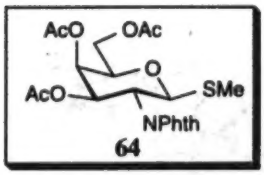
Octyl 4-O-(2-acetamido-2,3-dideoxy-β-D-xylopyranosyl)-β-D-galactopynoside (**63**).



63

NaOMe (41 mg) was added to a solution of compound **62** (130 mg, 0.156 mmol) in dry MeOH (15 mL) and then the mixture was stirred for 3 h with TLC indicating the reaction had gone to completion (R_f = 0.31, CH_2Cl_2-MeOH, 10:1). The solution was neutralized with Dowex 50W-X8 $[H]^+$ resin, filtered, and concentrated. The crude product was hydrogenolyzed over $Pd(OH)_2/C$ (20%, 30 mg) in ethanol (98%, 15 mL) for 3 h, filtered through Celite and concentrated. The resulting residue was purified by column chromatography (4:1, CH_2Cl_2-MeOH containing 1.5% of H_2O) to give the target compound **63** (68 mg, 91%; R_f = 0.36, 4:1, CH_2Cl_2-MeOH containing 1.5% of H_2O; $[\alpha]_D$ = - 24°, *c* = 0.8, in MeOH). Selected NMR (500 MHz, CD_3OD): ^1H: δ = 4.64 (d, 1 H, J = 8.7 Hz, H-1'), 4.19 (d, 1 H, J = 7.8 Hz, H-1), 2.36 (m, 1 H, H-3e'), 1.57 (m, 1 H, H-3a'), and 1.95 (s, 3 H, NAc). ^{13}C: δ = 106.0 (C-1', $J_{C1'H1'}$ = 160.5 Hz) and 104.5 (C-1, J_{C1H1} = 158.2 Hz). FAB-MS: Calcd for $C_{22}H_{41}NO_{10}$ $[M+Na]^+$ and $[M+H]^+$: *m/z* 502 and 480. Found: *m/z* 502 and 480.

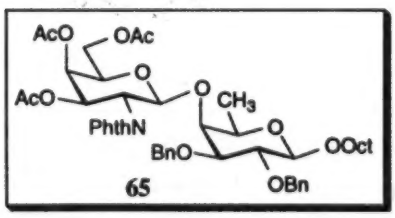
Methyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside (**64**).



64

To a solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranoside (3 g, 6.2 mmol) in 1,2-dichloroethane (125 mL), TMSSMe (2.7 mL, 18.9 mmol) and TMSOTf (1.85 mL, 9.3 mmol) were added at rt. The reaction temperature was increased to 40 °C and the solution was stirred for 45 h. Satd. $NaHCO_3$ (50 mL) and CH_2Cl_2 (100 mL) were added to the reaction mixture. The organic layer was dried with Na_2SO_4, filtered and concentrated. The resulting residue was passed through a silica column (hexane-EtOAc, 2:1) to give product **64** (2.8 g, 95%). NMR ($CDCl_3$): ^1H: δ = 7.9-7.7 (m, 4 H, aromatic), 5.86 (dd, 1 H, J = 11.0, 3.5 Hz), 5.51 (d, 1 H, J = 3.5 Hz, H-4), 5.34 (d, 1 H, J = 10.5 Hz, H-1), 4.62 (bt, 1 H, J = 11.0, 10.5 Hz, H-2), 4.15 (m, 3 H, H-5 and 2 x H-6), 2.05, 2.20 and 2.18 (3 s, 3 x OAc), and 1.85 (s, 3 H, SMe).

Octyl 4-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl)-2,3-di-O-benzyl-6-deoxy-β-D-galactopyranoside (**65**).

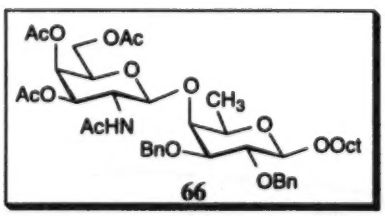


65

A solution of compound **46** (141 mg, 0.310 mmol), compound **64** (216 mg, 0.465 mmol) and AW-300 molecular sieves (powder, 300 mg) in dry MeCN (4 mL) and dry CH_2Cl_2 (1.2 mL) was stirred for about 30 min at rt under Ar and cooled to - 50 °C. To the cooled mixture, a solution of NIS (140 mg, 0.620 mmol) and TfOH (5.5 μL, 0.062 mmol) in dry MeCN (0.3 mL) was added. After 30 min, the solution was diluted with CH_2Cl_2 (20 ml), neutralized with Et_3N (2 mL) and filtered. The resulting filtrate was successively washed with aq $Na_2S_2O_3$ and satd $NaHCO_3$, dried with Na_2SO_4, filtered and then concentrated.

The residue was chromatographed on a silica gel column (hexane-EtOAc, 3:1) to afford compound **65** (218 mg, 81%; R_f = 0.35, hexane-EtOAc, 2:1; $[\alpha]_D$ = - 15°, *c* = 0.7, in $CHCl_3$). NMR ($CDCl_3$): ^1H: δ = 7.90-7.00 (m, 14 H, aromatics), 6.10 (dd, 1 H, J = 11.5, 3.4 Hz, H-3'), 5.49 (d, 1 H, J = 3.4 Hz, H-4'), 5.29 (d, 1H, J = 8.4 Hz, H-1'), 4.68 (dd, 1 H, J = 11.5, 8.4 Hz, H-2'), 4.45 and 3.60 (2 d, 2 H, J = 10.5 Hz, O*CH*$_2$Ph), 4.33 and 4.10 (2 d, 2 H, J = 12.5 Hz, O*CH*$_2$Ph), 4.20-4.02 (m, 3 H, H-5' and 2 x H-6'), 4.12 (d, 1 H, J = 7.5 Hz, H-1), 3.86 (dt, 1 H, J = 9.5, 6.5 Hz, O*CH*$(CH_2)_6CH_3$), 3.36 (dt, 1 H, J = 9.5, 7.0 Hz, O*CH*$(CH_2)_6CH_3$), 3.30 (d, 1 H, J = 2.5 Hz, H-4), 3.29 (q, 1 H, J = 6.5, H-5), 3.16 (dd, 1 H, J = 9.5, 2.5 Hz, H-3), 3.08 (dd, 1 H, J = 9.5, 7.5 Hz, H-2), 2.15, 2.05 and 1.88 (3 s, 9 H, 3 x OAc), 1.58 (m, 2 H, $OCH_2CH_2(CH_2)_5CH_3$), 1.25 (bs, 10 H, $O(CH_2)_2(CH_2)_5CH_3$), 1.22 (d, 3 H, J = 6.5 Hz, 3 x H-6), and 0.87 (t, 3 H, $O(CH_2)_7CH_3$). ^{13}C: δ = 170.45, 169.94, 168.55, 167.57, 138.53, 138.22, 133.82, 133.68, 132.56, 131.83, 128.53, 128.33, 128.15, 127.90, 127.79, 127.48, 123.31, 123.14, 103.20, 99.91, 80.14, 79.89, 79.60, 75.01, 72.90, 70.09, 69.74, 69.53, 67.93, 66.61, 61.56, 51.37, 31.87, 29.72, 29.48, 29.27, 26.20, 22.69, 20.72, 20.64, 16.80, and 14.11. Anal. Calcd for $C_{48}H_{59}NO_{14}$: C, 65.96; H, 6.80; N, 1.60. Found: C, 66.18; H, 6.94; N, 1.49.

Octyl 4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-2,3-di-O-benzyl-6-deoxy-β-D-galactopyranoside (**66**).

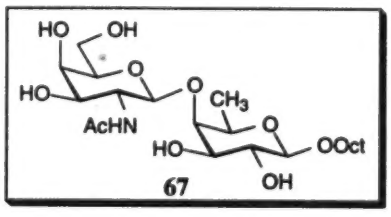


66

A solution of compound **65** (175 mg, 0.200 mmol) and hydrazine monohydrate (1.0 mL) in ethanol (98%, 10 mL) was refluxed at 90 °C (oil bath temperature) for 1 h and then concentrated, co-evaporated with ethanol three times to remove the excess of hydrazine followed by co-evaporation with toluene twice. The residue was dissolved in pyridine-Ac_2O (1:1, 10 mL)

and the resulting solution was kept overnight at rt, concentrated and co-evaporated with toluene. The residue was applied to a silica gel column (hexane-EtOAc, 1:1) to give compound **66** (141 mg, 90%; R_f = 0.34, hexane-EtOAc, 1:1; $[\alpha]_D$ = + 4°, *c* = 0.7, in $CHCl_3$). NMR ($CDCl_3$): ^1H: δ = 7.45-7.25 (m, 15 H, aromatics), 5.32 (bd, 1 H, J = 3.0 Hz, H-4'), 5.15 (dd, 1 H, J = 11.0, 3.2 Hz, H-3'), 4.91 and 4.65 (2 d, 2 H, J = 11.5 Hz, O*CH*$_2$Ph), 4.88 (d, 1 H, J = 8.5 Hz, H-1'), 4.74 and 4.69 (2 d, 2 H, J = 11.0 Hz, O*CH*$_2$Ph), 4.32 (d, 1 H, J = 7.5 Hz, H-1), 4.12 (d, 2 H, J = 6.5 Hz, 2 x H-6'), 3.97 (bt, 1 H, J = 6.5 Hz, H-5'), 3.95 (dd, 1 H, J = 11.0, 8.5 Hz, H-2'), 3.85 (dt, 1 H, J = 9.5, 6.0 Hz, O*CH*$(CH_2)_6CH_3$), 3.82 (bd, 1 H, J = 1.5 Hz, H-4), 3.65 (td, 1 H, J = 10.0, 2.5 Hz, H-3), 3.60-3.51 (bm, 1 H, H-5), 3.49 (dd, 1 H, J = 10.0, 7.5 Hz, H-2), 3.48 (dt, 1 H, J = 9.5, 7.0 Hz, O*CH*$(CH_2)_6CH_3$), 2.13, 2.02 and 1.92 (3 s, 9 H, 3 x OAc), 1.76 (s, 3 H, NAc), 1.57 (bm, 2 H, $OCH_2CH_2(CH_2)_5CH_3$), 1.25 (bs, 10 H, $O(CH_2)_2(CH_2)_5CH_3$), 1.23 (d, 3 H, 3 x H-6), and 0.86 (t, 3 H, $O(CH_2)_7CH_3$). ^{13}C: δ = 173.34, 172.13, 172.00, 171.69, 140.28, 139.61, 133.89, 129.76, 129.25, 129.14, 128.60, 128.51, 126.53, 104.81, 102.83, 82.63, 80.82, 78.62, 76.09, 74.33, 72.25, 71.66, 70.97, 70.71, 68.18, 62.73, 52.47, 32.92, 30.87, 30.47, 30.36, 27.30, 23.67, 23.24, 20.63, 17.26, and 14.43. Anal. Calcd for $C_{42}H_{59}NO_{13}$: C, 64.19; H, 7.57; N, 1.78. Found: C, 64.10; H, 7.62; N, 1.75.

Octyl 4-O-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-6-deoxy-β-D-galactopyranoside (**67**).

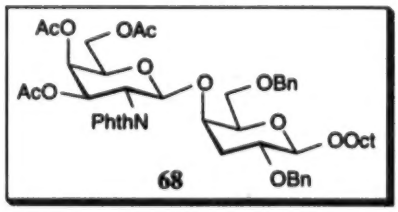


67

Compound **66** (130 mg, 0.166 mmol) was hydrogenolyzed over Pearlman's catalyst (20%, 30 mg) in ethanol (98%, 15 mL) overnight. The mixture was filtered through a Celite pad. The filtrate was concentrated and co-evaporated with toluene three times. The residue was dissolved in dry

MeOH (10 mL) and NaOMe (27 mg) was added. The resulting mixture was stirred overnight and then neutralized with Dowex 50W-X8 $[H]^+$ resin, filtered, and concentrated. The residue was purified by column chromatography (4:1, CH_2Cl_2-MeOH containing 1.5% of H_2O) to give compound **67** (67.5 mg, 85%; R_f = 0.29, 4:1, CH_2Cl_2-MeOH containing 1.5% of H_2O; $[\alpha]_D$ = - 14°, *c* = 0.6, in MeOH). Selected NMR (500 MHz, CD_3OD): ^1H: δ = 4.69 (d, 1 H, J = 8.4 Hz, H-1'), 4.34 (d, 1 H, J = 7.9 Hz, H-1), 2.06 (s, 3 H, NAc), and 1.28 (d, 3 H, 3 x H-6). ^{13}C: δ = 103.55 (C-1, J_{C1H1} = 160.3 Hz) and 103.25 (C-1', $J_{C1'H1'}$ = 163.0 Hz). FAB-MS: Calcd for $C_{22}H_{41}NO_{10}$ $[M+Na]^+$ and $[M+H]^+$: *m/z* 502 and 480. Found: *m/z* 502 and 480.

Octyl 4-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl)-2,6-di-O-benzyl-3-deoxy-β-D-xylopyranoside (**68**).

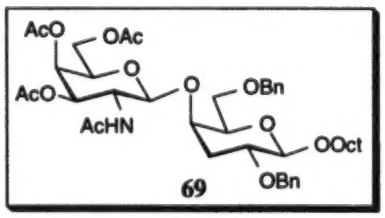


68

A mixture of **49** (200 mg, 0.438 mmol), **64** (346 mg, 0.745 mmol) and 4 Å molecular sieves (powder, 2 g) in dry CH_2Cl_2 (20 mL) was stirred for about 30 min at rt under Ar and cooled to - 25 °C. To the cooled mixture, a solution of NIS (168 mg, 0.745 mmol) and TfOH (6.6 μL, 0.075 mmol) in dry CH_2Cl_2 (2 mL) was added. After 2 h, the solution was diluted with CH_2Cl_2, neutralized with Et_3N and filtered. The filtrate was successively washed with aqueous $Na_2S_2O_3$ and satd $NaHCO_3$, dried with Na_2SO_4, and then concentrated. The residue was passed through a silica gel column (toluene-acetone, 12:1) to yield compound **68** (296 mg, 77%; R_f = 0.3, hexane-EtOAc, 2:1; $[\alpha]_D$ = - 32°, *c* = 0.7, in $CHCl_3$). NMR ($CDCl_3$): ^1H: δ = 7.90-7.05 (m, 14 H, aromatics), 5.75 (dd, 1 H, J = 11.5, 3.5 Hz, H-3'), 5.46 (d, 1 H, J = 8.2 Hz, H-1'), 5.47 (bd, 1 H, J = 3.0 Hz, H-4'), 4.57 (dd, 1 H, J = 11.5, 8.2 Hz, H-2'), 4.59 and 4.55 (2 d, 2 H, J = 12.5 Hz, O*CH*$_2$Ph), 4.41 and 3.71 (2 d, 2 H, J = 11.5 Hz, O*CH*$_2$Ph), 4.22 (d, 1 H, J = 7.8 Hz, H-1), 4.17 (dd, 1 H, J =

11.5, 7.0 Hz, H-6a'), 4.08 (dd, 1 H, J = 11.5, 6.0 Hz, H-6b'), 4.04 (btd, 1 H, J = 6.5, 1.0 Hz, H-5'), 3.97 (bs, 1 H, H-4), 3.80 (dt, 1 H, J = 9.5, 6.5 Hz, O*CH*$(CH_2)_6CH_3$), 3.76 and 3.66 (m, 3 H, H-5, and 2 x H-6), 3.38 (dt, 1 H, J = 9.5, 7.0 Hz, O*CH*$(CH_2)_6CH_3$), 2.95 (ddd, 1 H, J = 11.5, 7.8, 4.8 Hz, H-2), 2.10 (ddd, 1 H, J = 14.5, 4.8, 3.2 Hz, H-3e), 2.03, 1.86 and 1.58 (3 s, 9 H, 3 x OAc), 1.53 (m, 2 H, $OCH_2CH_2(CH_2)_5CH_3$), 1.45 (ddd, 1 H, J = 14.5, 11.5, 2.8 Hz, H-3a), 1.25 (bs, 10 H, $O(CH_2)_2(CH_2)_5CH_3$), and 0.87 (t, 3 H, $O(CH_2)_7CH_3$). ^{13}C: δ = 170.36, 169.78, 168.38, 138.71, 138.52, 134.36, 131.345, 128.40, 128.22, 127.58, 127.39, 127.23, 123.77, 123.48, 104.96, 96.90, 76.30, 74.06, 73.54, 73.43, 73.04, 70.91, 69.81, 69.33, 68.34, 66.76, 61.33, 51.59, 33.52, 31.83, 29.65, 29.,41, 29.26, 26.10, 22.67, 20.76, 20.67, 20.53, and 14.10. Anal. Calcd for $C_{48}H_{59}NO_{14}$: C, 65.96; H, 6.80; N, 1.60. Found: C, 65.99; H, 6.92; N, 1.57.

Octyl 4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-2,6-di-O-benzyl-3-deoxy-β-D-xylopyranoside (**69**).

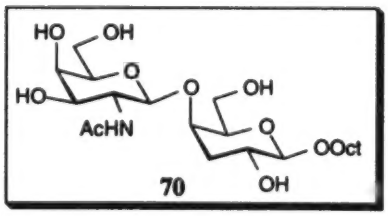


69

A solution of compound **68** (87 mg, 0.100 mmol) and hydrazine monohydrate (0.5 mL) in ethanol (98%, 5 mL) was refluxed at 90 °C (oil bath temperature) for 1 h, at which time the reaction had gone to completion as indicated by TLC (R_f = 0.15, CH_2Cl_2-MeOH, 10:1). The solution was concentrated and co-evaporated with ethanol three times followed by co-evaporation with toluene twice. The residue was dissolved in pyridine-Ac_2O (1:1, 10 mL). The solution was kept overnight at rt, concentrated and co-evaporated with toluene. The residue was applied to a silica gel column (hexane-EtOAc, 2:3) to give compound **69** (69.5 mg, 89%; R_f = 0.15, hexane-EtOAc, 1:1; $[\alpha]_D$ = - 23°, *c* = 0.8, in $CHCl_3$). NMR ($CDCl_3$): ^1H: δ = 7.40-7.30 (m, 10 H, aromatics), 5.49 (dd, 1 H, J = 11.5, 3.5 Hz, H-

3'), 5.36 (bd, 1 H, J = 3.5 Hz, H-4'), 5.28 (d, 1 H, J = 7.9 Hz, *NH*), 4.95 (d, 1 H, J = 8.3 Hz, H-1'), 4.86 and 4.62, 4.60 and 4.55 (4 d, 4 H, J = 12.0 Hz, 2 x O*CH*$_2$Ph), 4.08 (d, 1 H, J = 7.5 Hz, H-1), 4.10 (dd, 1 H, J = 11.0, 7.0 Hz, H-6a'), 4.05 (dd, 1 H, J = 11.0, 6.0 Hz, H-6b'), 4.00 (bs, 1 H, H-5'), 3.95 (dt, 1 H, J = 9.5, 6.5 Hz, O*CH*(CH$_2$)$_6$CH$_3$), 3.85 (bt, 1 H, J = 7.0 Hz, H-5), 3.68 (m, 2 H, H-4 and 2 x H-6), 3.55 (dt, 1 H, J = 11.5, 8.2 Hz, H-2'), 3.50 (dt, 1 H, J = 9.5, 7.0 Hz, O*CH*(CH$_2$)$_6$CH$_3$), 3.40 (ddd, 1 H, J = 11.5, 7.5, 5.0 Hz, H-2), 2.28 (ddd, 1 H, J = 14.5, 5.0, 3.0 Hz, H-3e), 2.22, 2.05 and 2.00 (3 s, 9 H, 3 x OAc), 1.88 (s, 3 H, NAc), 1.55 (ddd, 1 H, J = 14.5, 11.5, 2.5 Hz, H-3a), 1.65 (m, 2 H, OCH$_2$*CH$_2$*(CH$_2$)$_5$CH$_3$), 1.30 (bs, 10 H, O(CH$_2$)$_2$*(CH$_2$)$_5$*CH$_3$), and 0.88 (t, 3 H, O(CH$_2$)$_7$*CH$_3$*). ^{13}C: δ = 170.30, 170.13, 170.00, 138.81, 138.44, 128.64, 128.31, 127.60, 127.48, 127.01, 105.16, 97.59, 76.37, 73.44, 72.93, 72.24, 70.49, 69.81, 69.45, 69.02, 66.78, 61.36, 52.48, 33.83, 33.08, 31.74, 29.68, 29.35, 29.19, 26.12, 23.27, 22.58, 20.59, and 14.02. Anal. Calcd for $C_{42}H_{59}NO_{13}$: C, 64.19; H, 7.57; N, 1.78. Found: C, 64.00; H, 7.60; N, 1.77.

Octyl 4-O-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-3-deoxy-β-D-xylopynoside (**70**).

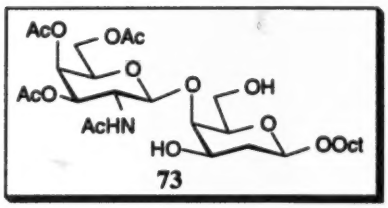


70

NaOMe (27 mg) was added to a solution of compound **69** (130 mg, 0.166 mmol) in dry MeOH (10 mL) and the resulting mixture was stirred for 1 h. TLC indicated the absence of starting material (R_f = 0.28, CH_2Cl_2-MeOH, 10:1). The solution was neutralized with Dowex 50W-X8 $[H]^+$ resin, filtered, and concentrated. The crude product was hydrogenolyzed over $Pd(OH)_2/C$ (20%, 26 mg) in ethanol (98%, 15 mL) for three hour, filtered through a Celite pad and concentrated. The residue was purified by column chromatography (3:1, CH_2Cl_2-MeOH containing 1.5% of H_2O) to give compound **70** (48 mg, 60%; R_f = 0.21, 3:1, CH_2Cl_2-MeOH containing 1.5% of H_2O; $[\alpha]_D$ = - 26°, *c* = 0.5, in H_2O). Selected NMR (500

MHz, D_2O): ^1H: δ = 4.51 (d, 1 H, J = 8.4 Hz, H-1'), 4.39 (d, 1 H, J = 7.9 Hz, H-1), 2.34 (ddd, 1 H, H-3e), 2.07 (s, 3 H, NAc), and 1.64 (m, 1 H, H-3a). ^{13}C: δ = 105.2 (C-1, J_{C1H1} = 160.5 Hz) and 100.7 (C-1', $J_{C1'H1'}$ = 160.5 Hz). FAB-MS: Calcd for $C_{22}H_{41}NO_{10}$ $[M+Na]^+$ and $[M+H]^+$: *m/z* 502 and 480. Found: *m/z* 502 and 480.

Octyl 4-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl)-2-deoxy-β-D-lyxopyranoside (**73**).

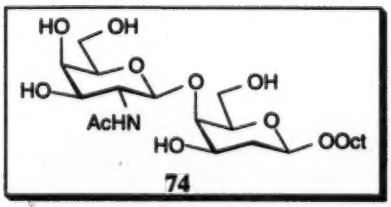


73

A mixture of **53** (103 mg, 0.226 mmol), **64** (157 mg, 0.339 mmol) and AW-300 molecular sieves (powder, 300 mg) in dry MeCN (3 mL) and dry CH_2Cl_2 (1.0 mL) was stirred for about 30 min at rt under Ar and cooled to - 50 °C. To the cooled mixture, a solution of NIS (102 mg, 0.452 mmol) and TfOH (2.0 μL, 0.023 mmol) in dry MeCN (0.3 mL) was added. After 30 min, TLC indicated complete reaction (R_f = 0.27, hexane-EtOAc, 2:1). The solution was diluted with CH_2Cl_2 (20 mL), neutralized with triethylamine (2 mL) and filtered. The filtrate was successively washed with aq $Na_2S_2O_3$ and satd $NaHCO_3$, dried with Na_2SO_4, and then concentrated. The residue was passed through a silica gel column (hexane-EtOAc, 2.5:1) to yield crude compound **71** (166 mg). A solution of crude **71** (122 mg) and hydrazine monohydrate (1.0 ml) in ethanol (98%, 10 mL) was refluxed for 1 h. TLC showed the absence of the crude **71** (R_f = 0.13, CH_2Cl_2-MeOH, 10:1). The solution was concentrated and co-evaporated with ethanol three times followed by co-evaporation with toluene twice. The residue was dissolved in pyridine-Ac_2O (1:1, 10 mL). The solution was kept overnight at rt, concentrated and co-evaporated with toluene. The resulting residue was applied to a silica gel column (hexane-EtOAc, 1:1) to give crude compound **72** (88 mg, 89%; R_f = 0.19, hexane-EtOAc, 1:1). Crude **72** (100 mg) was hydrogenolyzed

over Pearlman's catalyst (20%, 20 mg) in ethanol (98%, 10 mL) for 3 h. The mixture was filtered through Celite. The filtrate was concentrated and co-evaporated with toluene three times. The residue was purified by chromatography (CH_2Cl_2-MeOH, 20:1) to give compound **73** (31 mg, 28% overall yield; *Rf* = 0.51, CH_2Cl_2-MeOH, 10:1; $[\alpha]_D$ = - 10°, *c* = 0.7, in MeOH). NMR (CD_3OD): ^1H: δ = 5.13 (dd, 1 H, J = 3.3, 0.8 Hz, H-4'), 5.09 (dd, 1 H, J = 11.5, 3.3 Hz, H-3'), 4.85 (d, 1 H, J = 8.5 Hz, H-1'), 4.46 (dd, 1 H, J = 9.6, 3.3 Hz, H-1), 4.18 (dd, 1 H, J = 11.0, 6.0 Hz, H-6a'), 4.08 (dd, 1 H, J = 11.0, 7.0 Hz, H-6b'), 4.06 (dd, 1 H, J = 11.5, 8.5 Hz, H-2'), 4.01 (td, 1 H, J = 6.0, 0.8 Hz, H-5'), 3.86 (dt, 1 H, J = 9.5, 6.5 Hz, $OCH(CH_2)_6CH_3$), 3.85 (bs, 1 H, H-4), 3.82 (m, 1 H, H-3), 3.74 (dd, 1 H, J = 11.5, 6.0 Hz, H-6a), 3.68 (dd, 1 H, J = 11.5, 6.5 Hz, H-6b), 3.58 (s, 1 H, OH), 3.44 (dt, 1 H, J = 9.5, 7.0 Hz, $OCH(CH_2)_6CH_3$), 3.42 (bt, 1 H, J = 6.5 Hz, H-5), 3.34 (s, 1 H, *OH*), 2.15, 2.02 and 1.92 (3 s, 9 H, 3 x OAc), 1.91 (s, 3 H, NAc), 1.80 (bm, 1 H, H-2e), 1.71 (bm, 1 H, H-2a), 1.55 (bm, 2 H, $OCH_2CH_2(CH_2)_5CH_3$), 1.30 (bs, 10 H, $O(CH_2)_2(CH_2)_5CH_3$), and 0.88 (t, 3 H, $O(CH_2)_7CH_3$). ^{13}C: δ = 173.80, 172.16, 172.15, 171.79, 103.58, 101.63, 76.33, 76.17, 72.54, 72.03, 70.21, 70.14, 68.33, 64.32, 63.08, 62.73, 52.28, 36.32, 32.99, 31.37, 30.79, 30.53, 30.39, 27.18, 23.70, 23.10, 20.58, 20.55, and 14.41. Anal. Calcd for $C_{28}H_{47}NO_{13}$: C, 55.53; H, 7.82; N, 2.31. Found: C, 55.48; H, 7.90; N, 2.30.

Octyl 4-O-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-2-deoxy-β-D-galactopynoside (**74**).



74

Sodium methoxide (27 mg) was added to a solution of compound **73** (18 mg, 0.030 mmol) in dry MeOH (10 mL) and the mixture was stirred overnight. The solution was neutralized with Dowex 50W-X8 $[H]^+$ resin, filtered, and concentrated. The residue was purified by column

chromatography (4:1, CH_2Cl_2-MeOH containing 1.5% of H_2O) to give compound **74** (13.5 mg, 94%; R_f = 0.37, 4:1, CH_2Cl_2-MeOH containing 1.5% of H_2O; $[\alpha]_D$ = - 14°, *c* = 0.3, in MeOH). Selected NMR (500 MHz, CD_3OD): ^1H: δ = 4.51 (d, 1 H, J = 8.4 Hz, H-1'), 4.47 (dd, 1 H, J = 9.6, 2.1 Hz, H-1), 2.00 (s, 3 H, NAc). 1.84 (ddd, 1 H, H-2e), and 1.73 (dt, 1 H, H-2a), ^{13}C: δ = 103.85 (C-1', $J_{C1'H1'}$ = 161.3 Hz) and 101.30 (C-1, J_{C1H1} = 158.2 Hz). FAB-MS: Calcd for $C_{22}H_{41}NO_{10}$ $[M+Na]^+$ and $[M+H]^+$: *m/z* 502 and 480. Found: *m/z* 502 and 480.

Chapter 3

Synthesis of Simple Multivalent β-D-GalNAc-(1→4)-β-D-Gal Oligomers as Probes for Investigating the Interaction of *P. aeruginosa* Pili with Multivalent Receptors

3.1. Introduction.

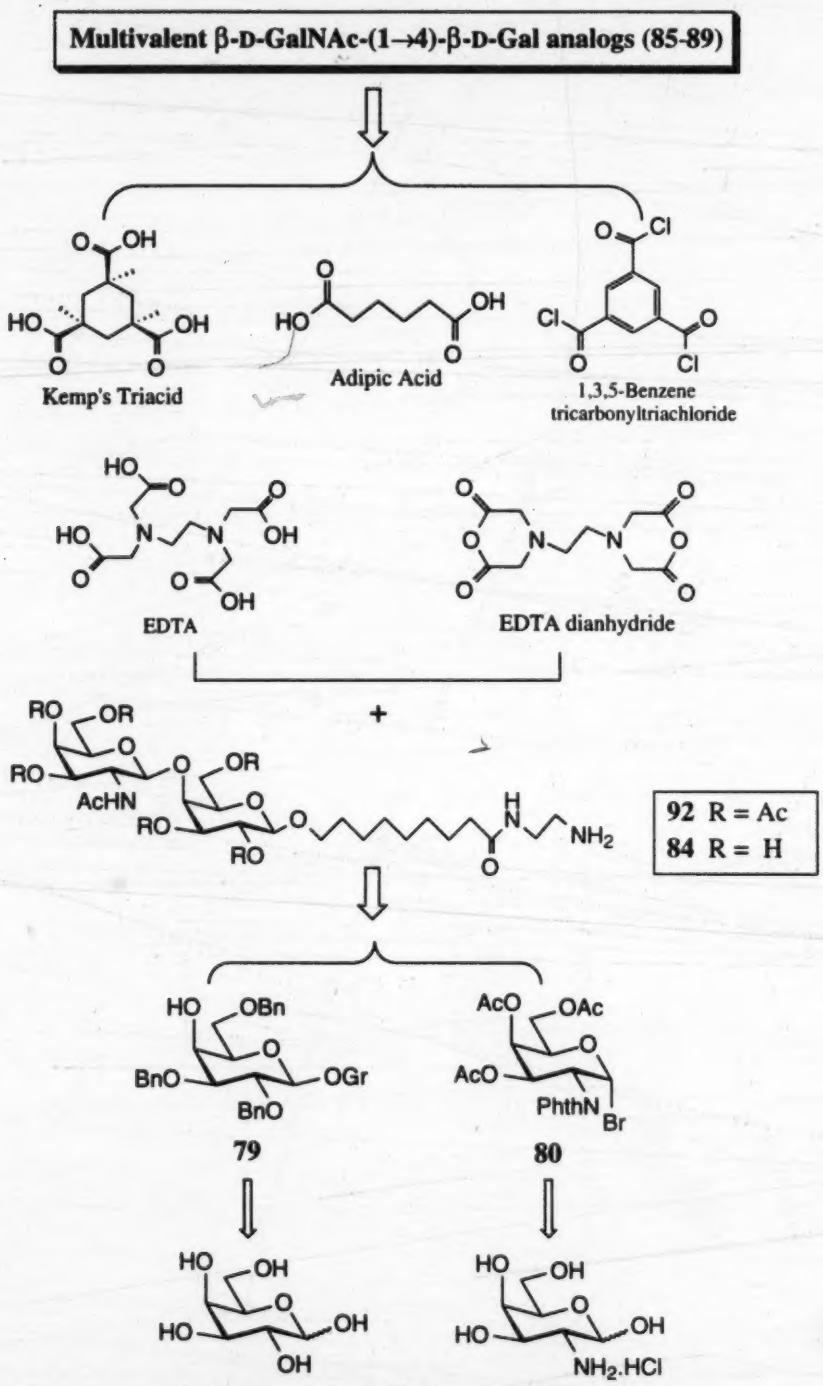
Microbial adherence to the cell surface is a key step in the initial stage of the infection process. Adhesins are structures on microbial surfaces which are used for adherence to host cells. *Pseudomoas aeruginosa* is an opportunistic pathogen which employs adhesins, called fimbriae or pili, to mediate attachment to host epithelial cells [24b, 30a, 81] and initiate many infections and diseases [82]. Previous studies have shown that pili adhesins on *P. aeruginosa* interact with the glycosphingolipid asialo-GM_1 receptor via its internal disaccharide sequence β-D-GalNAc-(1→4)-β-D-Gal, which is therefore suggested to play a crucial role in pili mediated adhesion of *P. aeruginosa* [34a, 35, 36a, 37, 38, 83]. In order to gain a more detailed understanding of the pilus-carbohydrate interaction, a chemical mapping approach employing single hydroxy-modified octyl β-D-GalNAc-(1→4)-β-D-Gal disaccharide analogs was initiated [40, 84]. In this study, individual hydroxy groups were replaced by a hydrogen, a methoxy group or a propyloxy group. Such a study is particularly useful to produce an understanding of the pilus-carbohydrate interaction and for developing inhibitors of adhesins that are simpler and have

higher affinity than the natural oligosaccharide ligands [85]. The research reported in the earlier part (Chapter 2) of this thesis involved the interaction of monovalent carbohydrates with the adhesins. Because of the multivalent nature of *P. aeruginosa*, it is expected that multivalent saccharides would bind to the cell surface adhesins more tightly than monovalent ones.

The advantage of using multivalent inhibitors arises from the fact that cell surfaces usually contain multiple receptor structures and adhesins possess multiple binding sites [46, 86]. Many adhesion processes mediated by protein-carbohydrate interactions employ multiple protein-carbohydrate complexes to provide the necessary avidity for tight binding to the cell surface [21a]. There has correspondingly been a great interest in the development of glycopolymers and dendrimers to achieve high-avidity binding [87]. To obtain a better insight into the nature of the receptor-adhesin interactions and more information for the design of anti-adhesive therapeutics, we therefore initiated the synthesis of simple readily accessible oligovalent saccharides. Simple templates were chosen, based on the need for eventual commercially viable inhibitors. Here, we report the synthesis of divalent (**85**, **86**), trivalent (**87**, **89**) and tetravalent (**88**) analogs of β-D-GalNAc-(1→4)-β-D-Gal.

3.1.1. Synthetic Strategy for Multivalent β-D-GalNAc-(1→4)-β-D-Gal Analogs.

In the synthesis of multivalent β-D-GalNAc-(1→4)-β-D-Gal analogs (Scheme 3.1), a C_9 spacer arm was chosed as the linker. This linking arm was developed by Lemieux *et al* [78] and has frequently been used to prepare artificial carbohydrate antigens via covalent attachment to protein. The required β-D-GalNAc-(1→4)-β-D-Gal disaccharide moiety was synthesized by the coupling of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-α-D-galactopyranosyl bromide with 2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-OMCO using



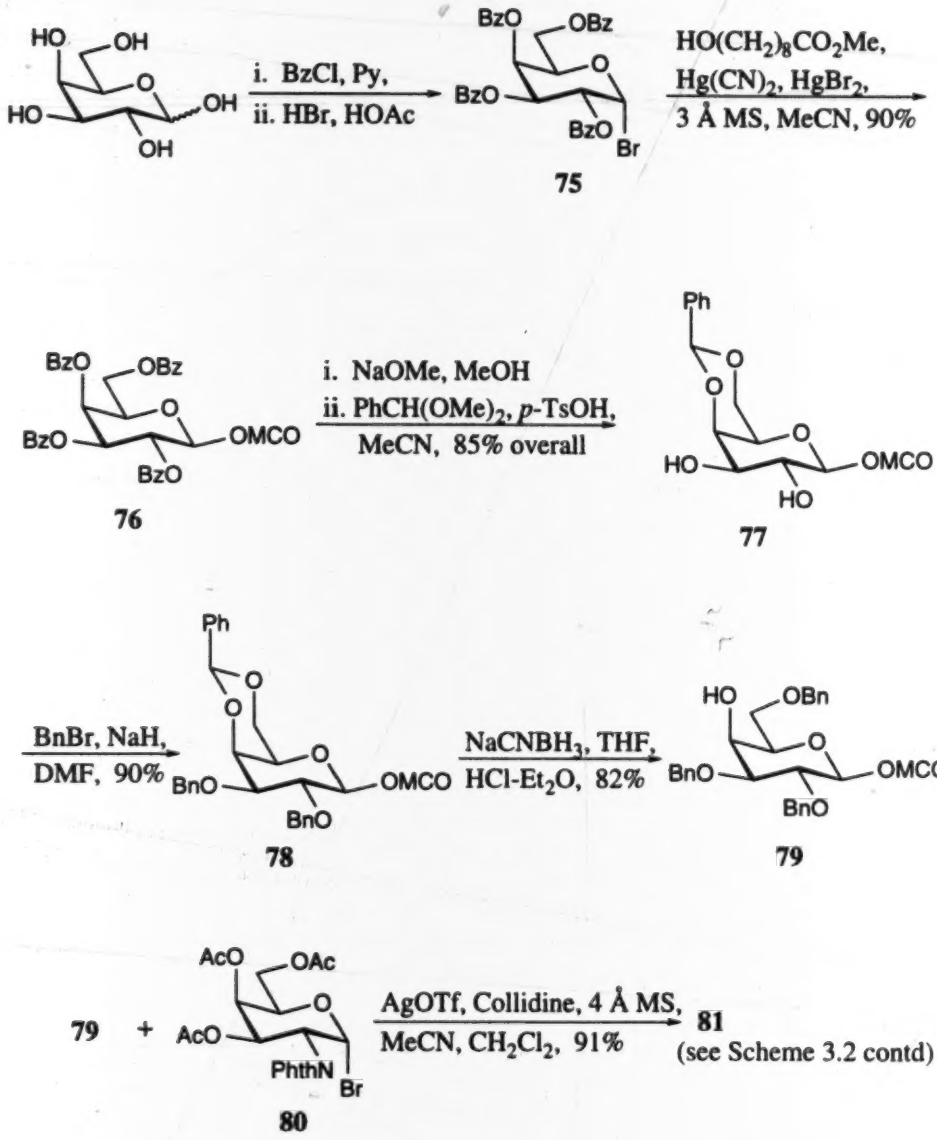
Scheme 3.1: Synthetic strategy for the preparation of multivalent β-D-GalNAc-(1→4)-β-D-Gal analogs.

AgOTf as promotor (Scheme 3.1). The β-D-GalNAc-(1→4)-β-D-Gal disaccharide with a C_9 spacer amine was used for coupling to various carboxylic acids, acid chlorides and anhydrides, such as Kemp's triacid, adipic acid, EDTA, 1,3,5-benzenetricarbonyltrichloride and EDTA dianhydride, using a varity of coupling reagents (for example, EDC, DIC and DCC) [79] under different reaction conditions. In this work, the hydrophilic moiety ethylenediaminetetraacetamide and hydrophobic moieties 1,3,5-benzenetriamide, 1,3,5-trimethyl-1,3,5-cyclohexanetriamide and adipamide were employed as cores for the multivalent template system. Ethylenediaminetetraacetamide and adipamide provide flexible linkers, while 1,3,5-benzenetriamide and 1,3,5-trimethyl-1,3,5-cyclohexanetriamide provide relatively rigid linkers. All of them can locate β-D-GalNAc-(1→4)-β-D-Gal moieties on one face of the molecule, offering the possibility of exploring multiple ligand binding. The various characteristics of the multivalent compounds allow for optimization of multivalent receptor-adhesin interactions.

3.1.2. Preparation of β-D-GalNAc-(1→4)-β-D-Gal with a C_9 Spacer Amine.

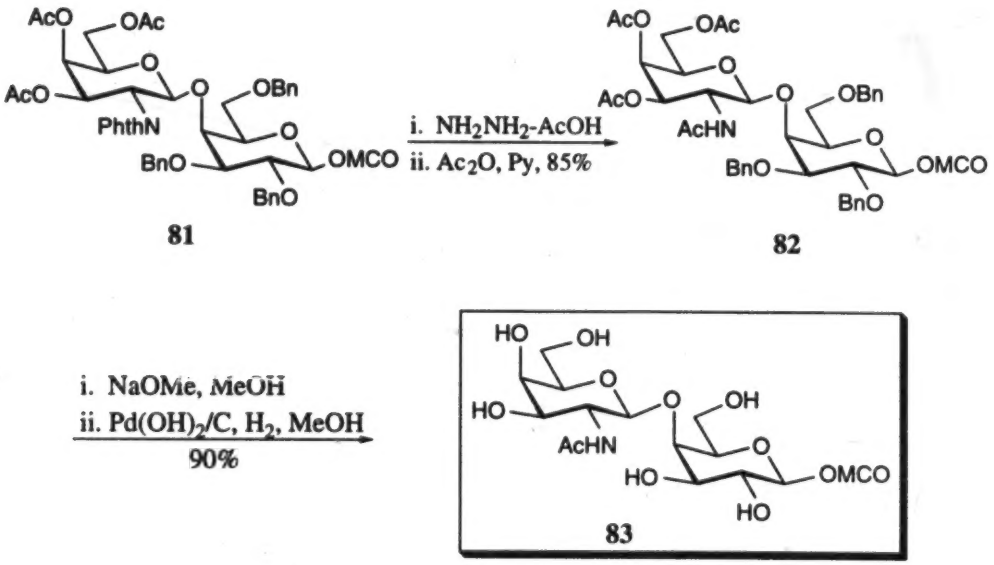
8-Methoxycarbonyloctyl 4-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-β-D-galactopyranoside (**83**) was synthesized as shown in Scheme 3.2. Benzoylation of D-galactose with benzoyl chloride in pyridine [80], followed by bromination using 33% HBr in AcOH gave the required bromide donor **75**. Coupling of **75** with 8-methoxycarbonyloctan-1-ol, in the presence of $Hg(CN)_2$ and $HgBr_2$ and 3 Å molecular sieves in dry MeCN, yielded **76** (90%). Debenzoylation of **76** with NaOMe in MeOH, followed by benzylidenation with benzaldehyde dimethyl acetal and *p*-TsOH in MeCN, gave **77** in 85% overall yield. Benzylation of **77** (BnBr and NaH in DMF at 0 °C to rt overnight) produced **78** (90%). Treatment of **78** with $NaCNBH_3$ and HCl-Et_2O in the presence of 3 Å molecular sieves in THF at 0 °C to rt resulted in benzylidene ring opening to give **79** in 82% yield. Glycosylation of **79** with 3,4,6-tri-*O*-acetyl-2-deoxy-2-

phthalimido-α-D-galactopyranosyl bromide (**80**) gave disaccharide **81** (91%). Removal of the phthalimido group in **81** using hydrazine acetate in refluxing MeOH overnight [81]



Scheme 3.2: Preparation of β-D-GalNAc-(1→4)-β-D-Gal-OMCO (**83**). (MCO = 8-methoxycarbonyloctyl).

followed by re-acetylation with Ac_2O-pyridine, gave **82** in 85% overall yield. Phthalimido groups in sugars are usually removed by refluxing in hydrazine-EtOH (1:10). Under such

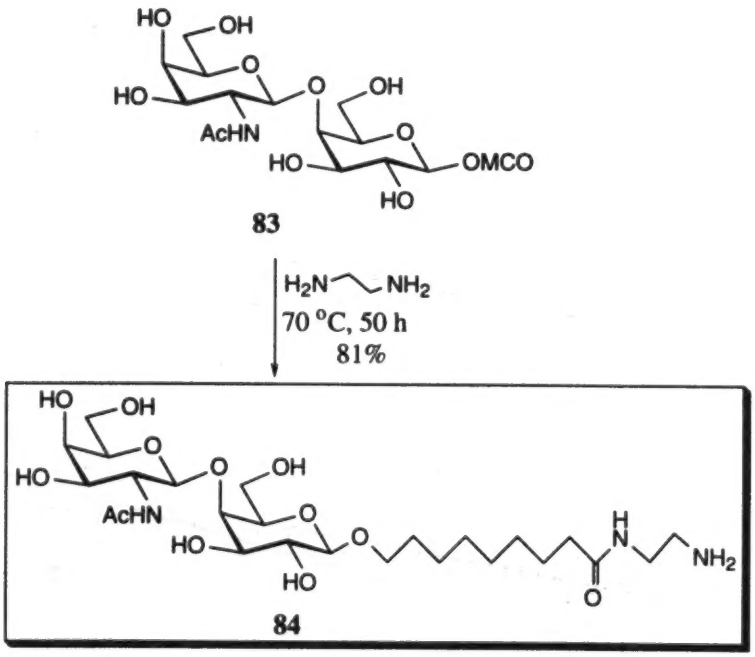


Scheme 3.2: Preparation of β-D-GalNAc-(1→4)-β-D-Gal-OMCO (**83**) (contd).

conditions, however, the methoxy group on the aglycon does not survive. Deacetylation of **82** with NaOMe in MeOH, followed by debenzylation using Pearlman's catalyst under hydrogen gas in methanol yielded **83** (90% overall yield). If hydrogenolysis was performed in EtOH, transesterification occurred to yield the ethyl ester.

The synthesis of β-D-GalNAc-(1→4)-β-D-Gal with an 8-(2-aminoethyleneamino-carbonyl)octyl aglycon (**84**) was performed as shown in Scheme 3.3. Treatment of **83** with neat anhydrous ethylenediamine at 70 °C for two days [82] and purification using

column chromatography (Iatrobeads) followed by adsorption on a C-18 Sap-Pak cartridge [91], washing with water and elution with methanol, gave **84** in 81% yield. The chromatography was essential to rid **84** of ethylenediamine which otherwise interfered with the subsequent amide coupling reactions. The ^{1}H NMR spectrum of **84** confirmed the

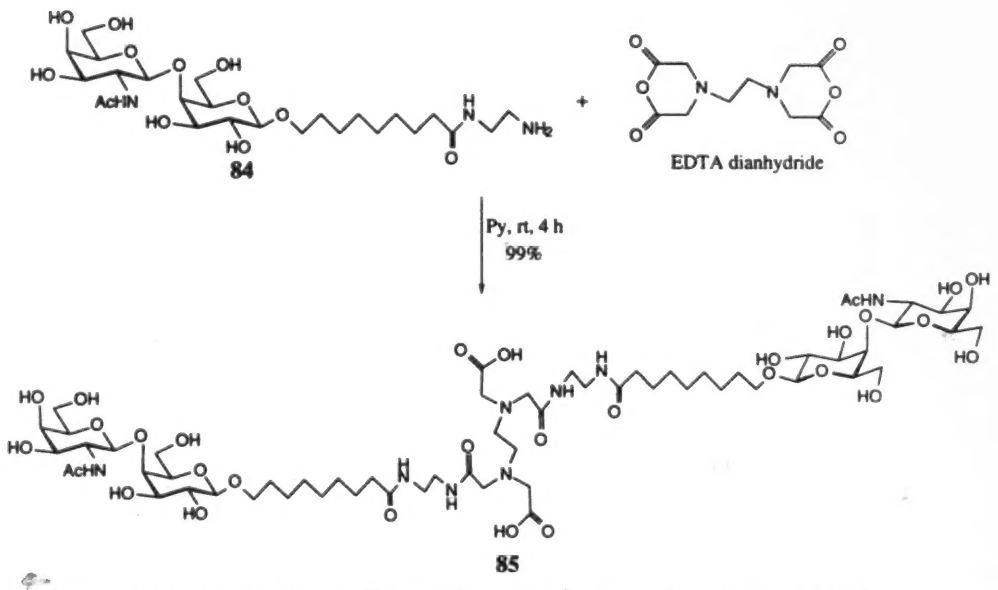


Scheme 3.3: Preparation of β-D-GalNAc-(1→4)-β-D-Gal with a C_9 spacer amine (**84**).

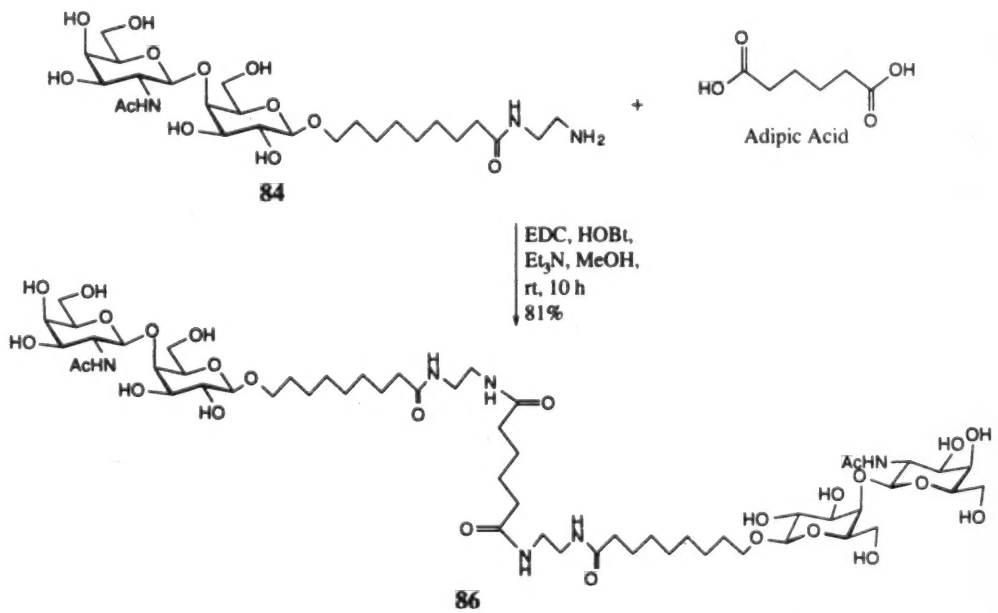
structure showing three triplet signals for CONH*CH*$_2$ (3.26 ppm, *J* 6.5 Hz), *CH*$_2$NH$_2$ (2.75 ppm, *J* 6.5 Hz) and *CH*$_2$CONH (2.20 ppm, *J* 7.5 Hz).

3.1.3. Synthesis of Multivalent β-D-GalNAc-(1→4)-β-D-Gal Analogs.

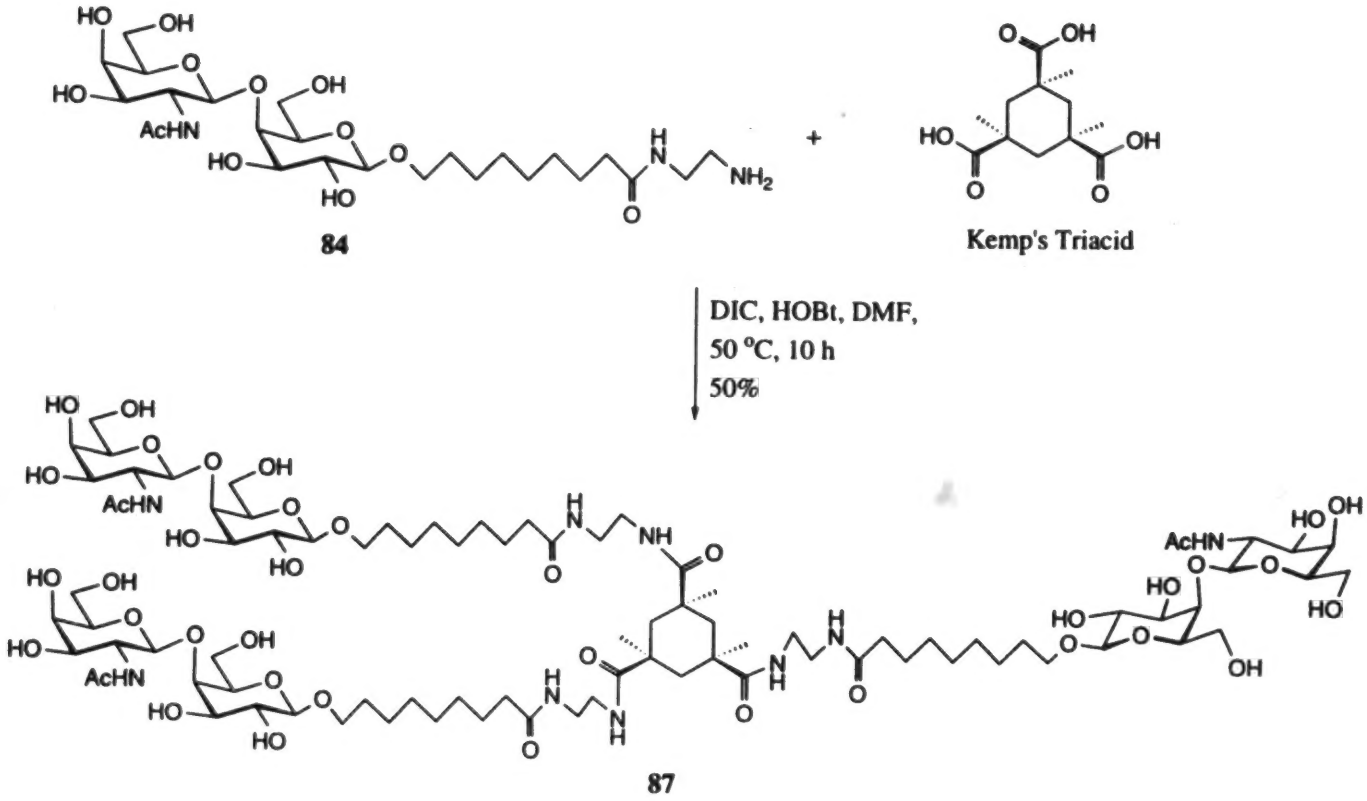
The synthesis of multivalent β-D-GalNAc-(1→4)-β-D-Gal analogs **85-88** was quite straightforward using coupling reactions of the amine **84** with carboxylic anhydrides or



Scheme 3.4: Synthesis of divalent β-D-GalNAc-(1→4)-β-D-Gal with an EDTA core (**85**).



Scheme 3.5: Synthesis of divalent β-D-GalNAc-(1→4)-β-D-Gal Adipic diamide (**86**).

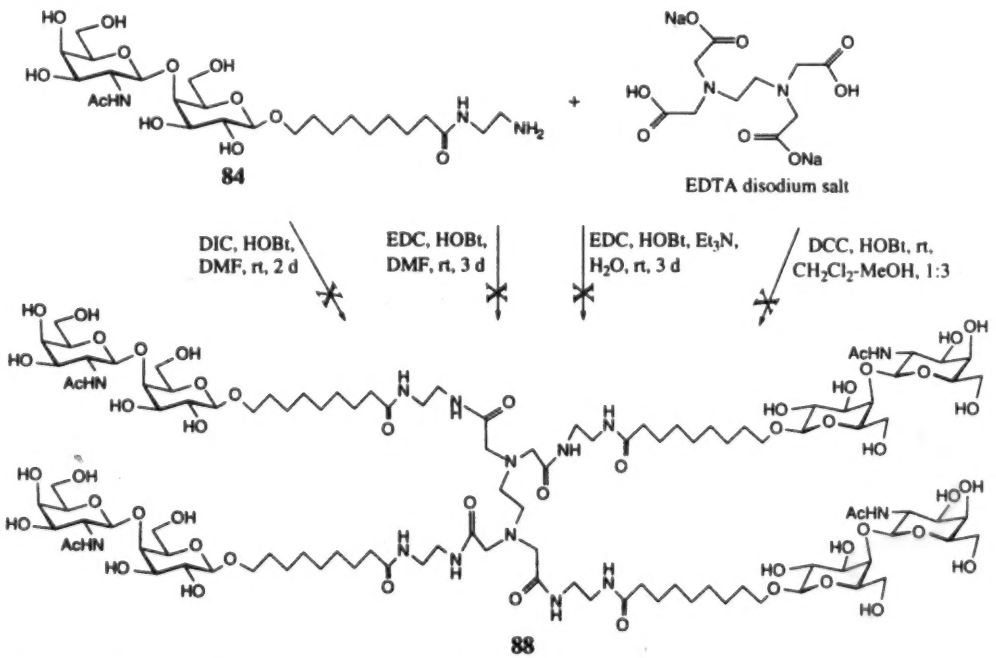


Scheme 3.6: Synthesis of trivalent β-D-GalNAc-(1→4)-β-D-Gal Kemp's triamide (**87**).

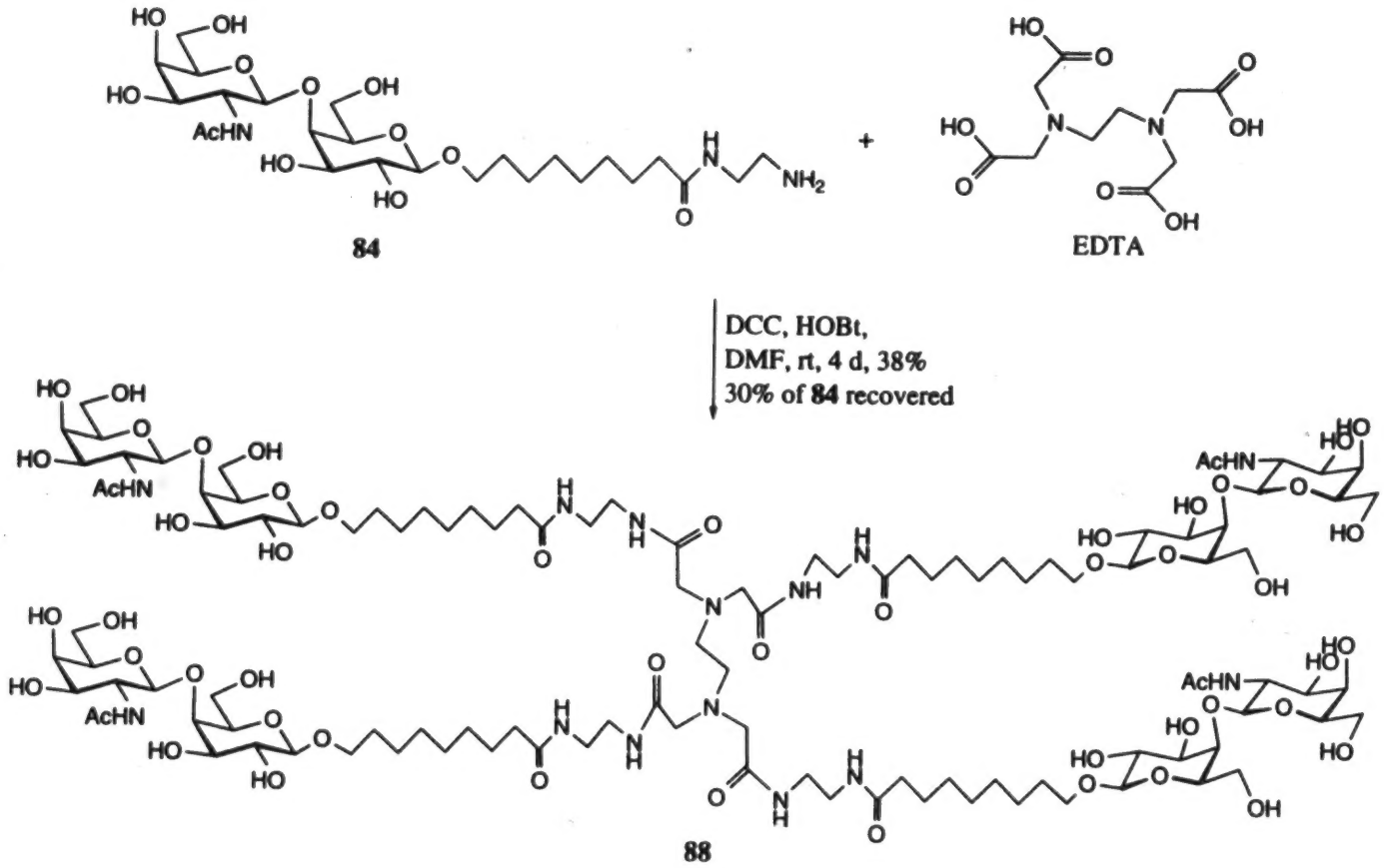
carboxylic acids. The disaccharide amine **84** was coupled with ethylenediaminetetraacetic dianhydride in dry pyridine at rt for 4 h to give the divalent β-D-GalNAc-(1→4)-β-D-Gal compound **85** in almost quantitative yield based on the dianhydride (Scheme 3.4) [83].

Compound **84** was treated with adipic acid in the presence of 1-[3-(dimethylamino)propyl]ethylcarbodiimide hydrochloride (EDC) [84] and 1-hydroxy-benzotriazole (HOBt) [85] as coupling reagents in Et_3N and MeOH. The reaction was performed at rt overnight and gave the divalent ligand **86** (81%) as a white powder (Scheme 3.5).

The use of *N,N'*-diisopropylcarbodiimide (DIC) [86] and HOBt in the coupling between **84** and Kemp's triacid in DMF at rt for 2 days did not give any trivalent compound **87**. However, heating the reaction mixture overnight at 50 °C gave **87** in 50% yield (Scheme 3.6).



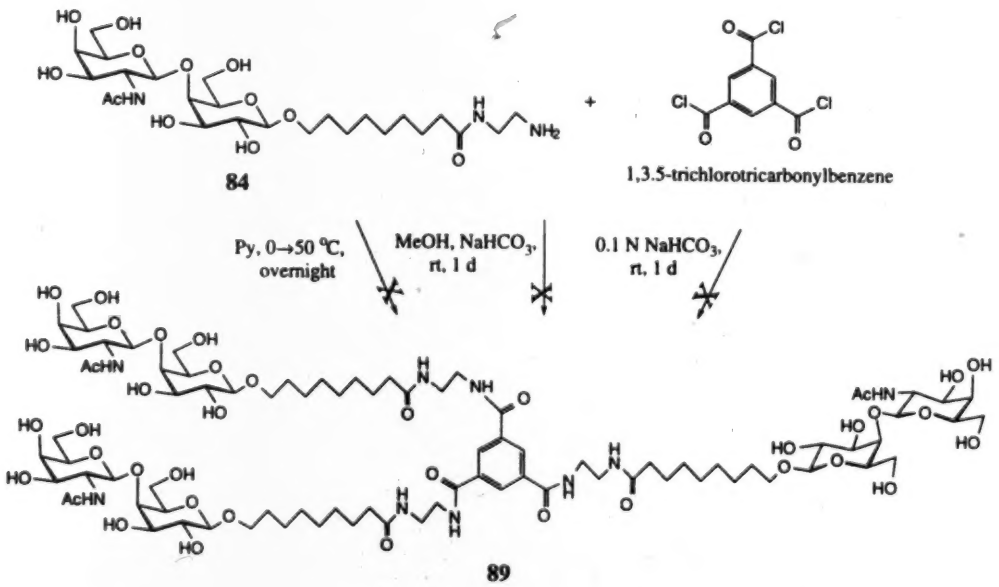
Scheme 3.7: Attempted synthesis of tetravalent β-D-GalNAc-(1→4)-β-D-Gal with an EDTA core (**88**).



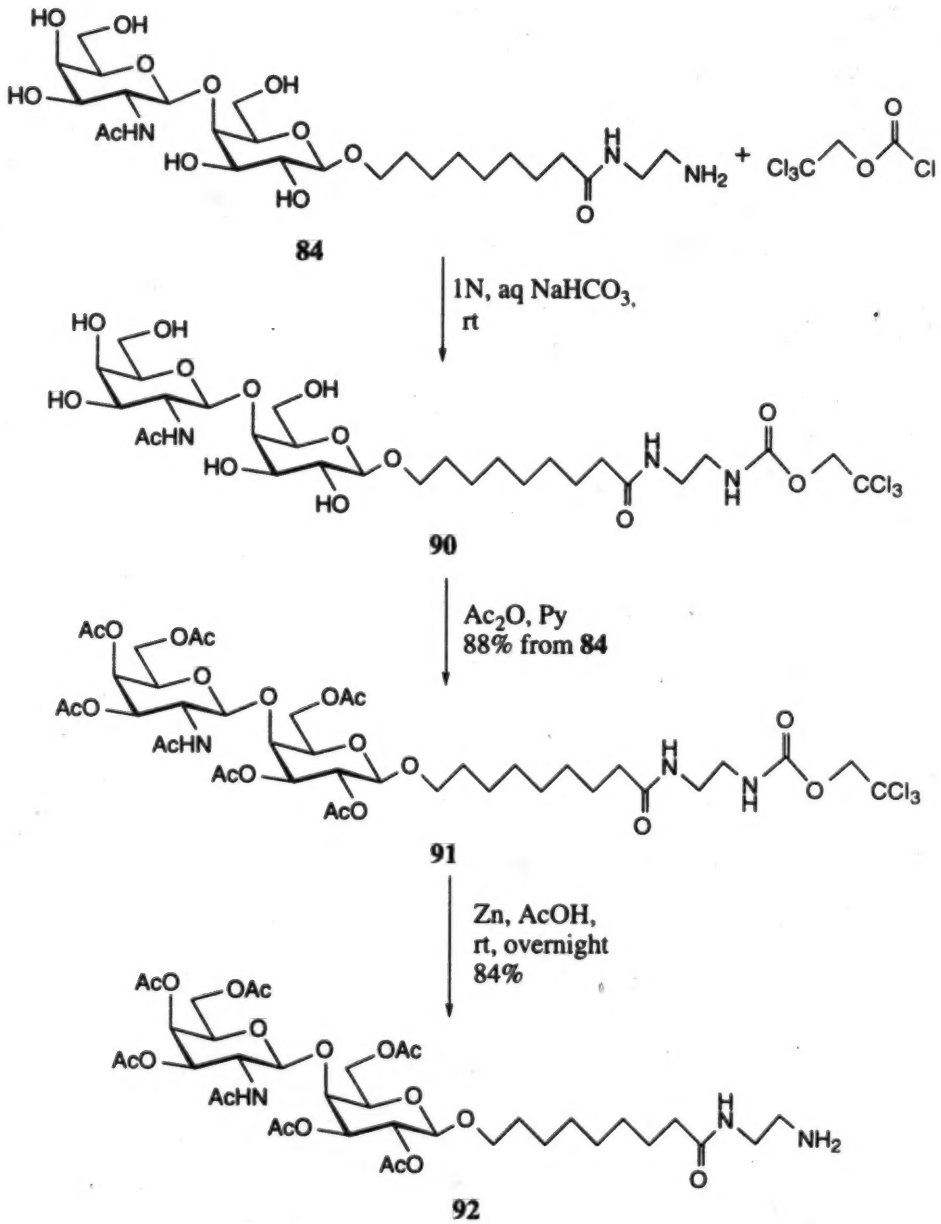
Scheme 3.8: Synthesis of tetravalent β-D-GalNAc-(1→4)-β-D-Gal with an EDTA core (**88**).

The synthesis of the tetravalent analog **88** was not so straightforward. Attempted reactions of divalent **85** with the disaccharide amine **84** using EDC and HOBt as coupling reagents failed. Similarly, reactions of EDTA with compound **84** using DIC and HOBt or EDC and HOBt as coupling reagents in different solvents (DMF, H_2O, CH_2Cl_2-MeOH) and at different temperatures did not produce compound **88** (Scheme 3.7). Finally, treatment of **84** with EDTA using 1,3-dicyclohexylcarbodiimide (DCC) [87] and HOBt in dry DMF at rt for 4 days finally furnished **88** as a white powder (38%, 30% starting material **84** recovered) (Scheme 3.8).

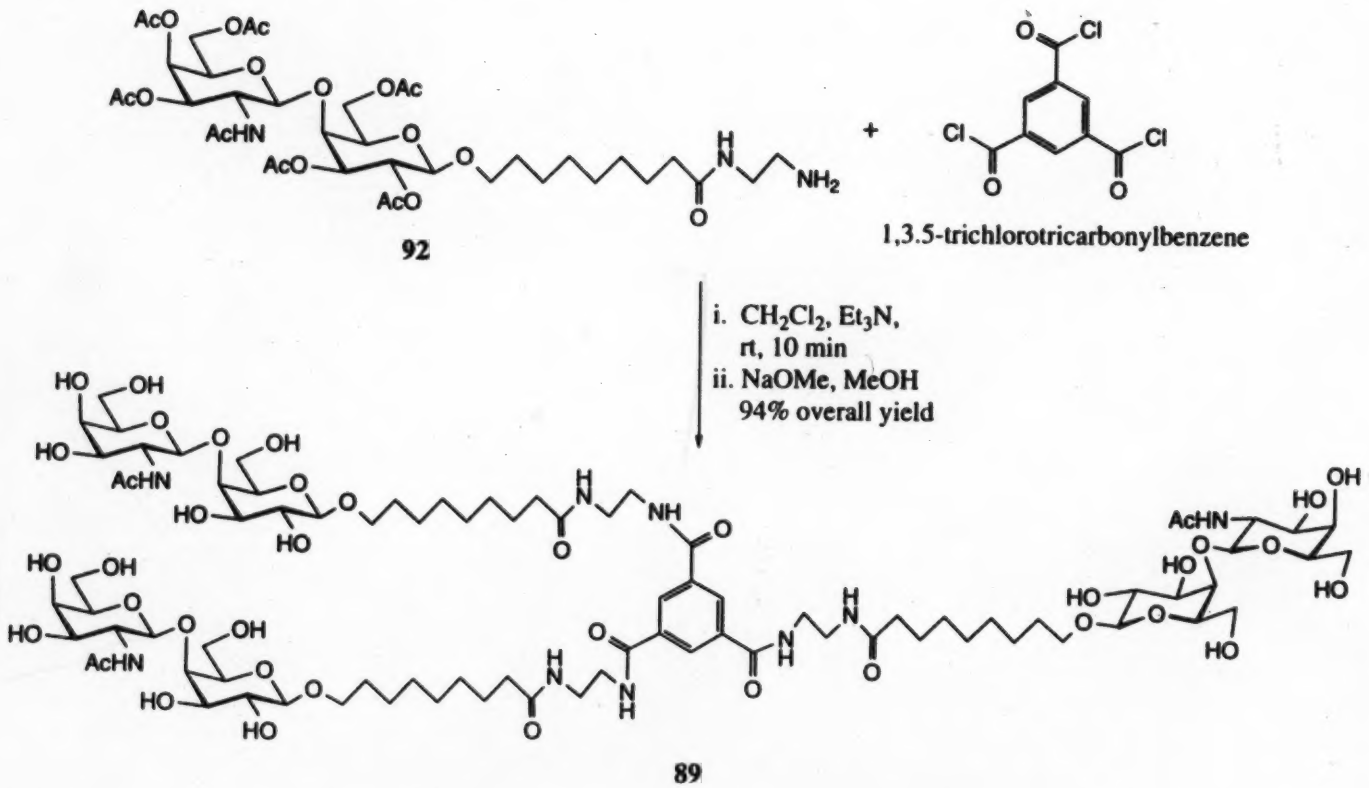
Attempted reactions of **84** with 1,3,5-benzenetricarbonyltrichloride in pyridine, in DMF and DMAP, in MeOH and $NaHCO_3$, or in 0.1 N aq $NaHCO_3$ failed to give the trivalent β-D-GalNAc-(1→4)-β-D-Gal analog **89** due to solubility problems (Scheme 3.9) [88]. The disaccharide **84** was therefore *O*-acetylated as shown in Scheme 3.10.



Scheme 3.9: Attempted synthesis of trivalent β-D-GalNAc-(1→4)-β-D-Gal 1,3,5-benzenetriamide **89**.



Scheme 3.10: Preparation of per-acetylated β-D-GalNAc-(1→4)-β-D-Gal with a C_9 spacer amine (**92**).



Scheme 3.11: Synthesis of trivalent β-D-GalNAc-(1→4)-β-D-Gal 1,3,5-benzenetriamide **89**.

Disaccharide amine **84** was treated with 2,2,2-trichloroethoxycarbonyl chloride (TrocCl) in 1N $NaHCO_3$ at rt [89] to yield the *N*-Troc product **90** in 97% yield. Pyridine could not be used as solvent because the hydroxy groups would then also react with TrocCl [90]. Acetylation of **90** with Ac_2O and pyridine at room temperature overnight gave acetylated disaccharide **91** (88%). The Troc group showed a sharp and characteristic two proton singlet at 4.75 ppm in the ^1H NMR spectrum. Compound **91** was stirred with zinc dust in glacial acetic acid at rt overnight to give the required acetylated amine **92** in 84% yield. This compound had to be utilized immediately to avoid inter-molecular *O*→*N* acetyl migration at rt.

Compound **92** was coupled with 1,3,5-benzenetricarbonyltrichloride in CH_2Cl_2 in the presence of triethylamine for 10 min to produce the trivalent acetylated β-D-GalNAc-(1→4)-β-D-Gal compound in quantitative yield. After deacetylation with NaOMe in MeOH at rt for 3 h, the target compound **89** was obtained in 94% overall yield (Scheme 3.11).

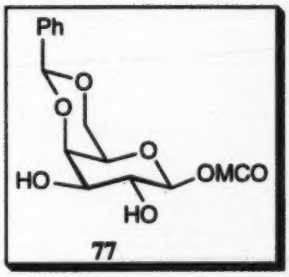
3.2. Experimental Section.

3.2.1. General Methods.

General methods were the same as described in Chapter 2, Part I.

3.2.2. Experimental

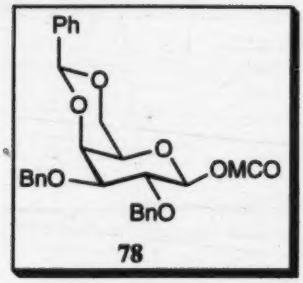
8-Methoxycarbonyloctyl 4,6,-O-benzylidene-β-D-galactopyranoside (**77**).



77

A mixture of bromide donor **75** (21.5 g, 33 mmol), grease alcohol (8.6 g, 46 mmol) and 3 Å molecular sieves (15 g) in dry MeCN (150 mL) was stirred for 1 h at rt. After cooled to 0 °C, $Hg(CN)_2$ (12.5 g, 50 mmol) and $HgBr_2$ (0.8 g) were added to the resulting mixture. TLC showed complete reaction after stirring 4 h at rt. Reaction solution was diluted with CH_2Cl_2 (300 mL), filtered, and concentrated. The residue was dissolved in CH_2Cl_2 (400 mL), washed with H_2O (300 mL), aq KI (10%, 300 mL) and satd $NaHCO_3$ (300 mL), dried, filtered and concentrated. Column purification (hexane-EtOAc, 3:1) of the residue gave **76** (90%). To a solution of **76** (20 g) in MeOH (200 mL) was added NaOMe (540 mL). After stirred 2 h, the solution was neutralized with Dowex 50-Wx8 [H^+] resin, filtered, concentrated and co-evaporated with toluene. The residue was benzylidenated with benzaldehyde dimethyl acetal (7.1 g, 57 mmol) and *p*-TsOH (100 mg) in MeCN (500 mL) overnight at rt. The resulting residue was neutralized with Et_3N, concentrated and then applied to a silica gel column (CH_2Cl_2-MeOH, 10:1) to give product **77** (85% overall yield). ^1H NMR ($CDCl_2$): δ = 7.55-7.3 (m, 5 H, aromatic), 5.51 (s, 1 H, Ph*CH*O_2), 4.34 (dd, 1 H, J = 12.5, 1.5 Hz, H-6a), 4.27 (d, 1 H, J = 7.4 Hz, H-1), 4.22 (dd, 1 H, J = 3.5, 1.0 Hz, H-4), 4.09 (dd, 1 H, J = 12.5, 1.8 Hz, H-6b), 3.97 (m, 1 H, O*CH*$(CH_2)_7$), 3.73 (m, 2 H, H-2 and H-3), 3.66 (s, 3 H, OMe), 3.50 (m, 2 H, H-5 and O*CH*$(CH_2)_7$), 2.5 (d, 2 H, 2 x *OH*), and 2.30 (t, 2 H, *CH_2*CO_2Me).

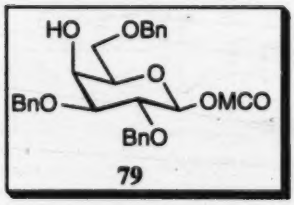
8-Methoxycarbonyloctyl 2,3-di-O-benzyl-4,6,-O-benzylidene-β-D-galactopyranoside (**78**).



78

A suspension of compound **77** (9 g, 20 mmol) and NaH (2.5 g, 80% in oil, 82 mmol) in dry DMF (140 mL) was stirred 15 min at rt and then cooled to about -5 °C. To the cooled suspension, BnBr (13 mL, 82 mmol) was added and the reaction mixture was warmed to rt and stirred overnight. To the reaction mixture was added MeOH (10 mL) to decompose the excess of NaH. The solution was diluted with EtOAc (500 mL), washed with Brine (3 x 200 mL), dried with $MgSO_4$ and concentrated. The resulting residue was passed through a silica gel column (hexane-EtOAc, 3:2) to give **78** (11.5 g, 90%). ^{1}H NMR ($CDCl_3$): δ = 7.60-7.3 (m, 15 H, aromatic), 5.50 (s, 1 H, Ph*CHO*$_2$), 4.93 and 4.77 (2 d, 2 H, J = 11.0 Hz, Ph*CH*$_2$O), 4.79 and 4.74 (2 d, 2 H, J = 12.5 Hz, Ph*CH*$_2$O), 4.38 (d, 1 H, J = 7.7 Hz, H-1), 4.30 (dd, 1 H, J = 12.5, 1.5 Hz, H-6a), 4.11 (d, 1 H, J = 3.5 Hz, H-4), 4.04-3.94 (m, 2 H, H-6b and O*CH*$(CH_2)_7$), 3.85 (dd, 1 H, J = 7.8, 10.0 Hz, H-2), 3.66 (s, 3 H, OMe), 3.55 (dd, 1 H, J = 3.5, 10.0 Hz, H-3), 3.44 (m, 1 H, O*CH*$(CH_2)_7$), 3.3 (bs, 1 H, H-5), and 2.30 (t, 2 H, *CH*$_2$$CO_2$Me).

8-Methoxycarbonyloctyl 2,3,6-tri-O-benzyl-β-D-galactopyranoside (**79**).

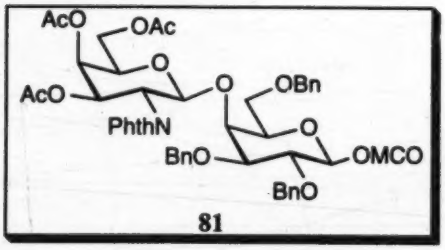


79

To a solution of compound **78** (11.0 g), $NaCNBH_3$ (20 g), 3 Å molecular sieves (powder, 20 g), and methyl orange (little) in THF (200 mL), satd. HCl in Et_2O was dropped in at 0 °C to keep the reaction solution in red color. The reaction was done after 4 h. The mixture was filtered and concentrated. The residue was re-dissolved in CH_2Cl_2 (500 mL), washed with H_2O, satd $NaHCO_3$, and H_2O, dried with $MgSO_4$, filtered and evaporated. After purified by chromatography (toluene-EtOAc, 5:1), product **79** was obtained (9.0 g, 82%). ^{1}H NMR ($CDCl_3$): δ = 7.45-7.25 (m, 15 H, aromatics), 4.92 and 4.75 (2 d, 2 H, J = 11.0 Hz, Ph*CH*$_2$O), 4.74 and 4.58 (2 s, 4 H, 2 x

Ph*CH*$_2$O), 4.34 (d, 1 H, J = 7.7 Hz, H-1), 4.02 (bt, 1 H, H-4), 3.94 (m, 1 H, O*CH*(CH$_2$)$_7$), 3.80 (dd, 1 H, J = 6.0, 10.0 Hz, H-6a), 3.72 (d, 1 H, J = 6.0, 10.0 Hz, H-6b), 3.63 (dd, 1 H, J = 7.7, 9.0 Hz, H-2), 3.66 (s, 3 H, OMe), 3.58-3.45 (m, 3 H, H-3, H-5 and O*CH*(CH$_2$)$_7$), 2.5 (d, 1 H, *OH*), and 2.30 (t, 2 H, *CH*$_2$CO$_2$Me)

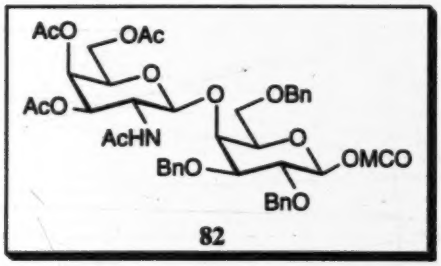
8-Methoxycarbonyloctyl 4-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl)-2,3,6-tri-O-benzyl-β-D-galactopyranoside (**81**).



81

A mixture of compound **79** (4.6 g, 7.4 mmol), bromide donor **80** (3.7 g, 7.4 mmol), collidine (0.68 mL, 5.2 mmol), and 4 Å molecular sieves (powder, 4 g) in toluene-MeNO$_2$ (1:1, 40 mL) was stirred for 1 h at rt and then cooled to -25 °C. To the cooled mixture, AgOTf (2.5 g, 9.6 mmol) was added. The reaction mixture was increased to rt and then kept overnight with stirring. The reaction solution was neutralized with Et$_3$N, filtered and evaporated. The resulting residue was purified with a silica gel column (toluene-EtOAc, 5:1) to give disaccharide **81** (7.0 g, 91%). ^{1}H NMR (CDCl$_3$): δ = 7.90-7.00 (m, 19 H, aromatics), 6.1 (dd, 1 H, J = 11.5, 3.5 Hz, H-3'), 5.50 (d, 1 H, J = 3.5, H-4'), 5.35 (d, 1 H, J = 8.5 Hz, H-1'), 4.65 (dd, 1 H, J = 11.5, 8.5 Hz, H-2'), 4.53 (s, 2 H, Ph*CH*$_2$O), 4.43 and 3.58 (2 d, 2 H, J = 10.5 Hz, Ph*CH*$_2$O), 4.36 and 4.19 (2 d, 2 H, J = 12.5 Hz, 2 x Ph*CH*$_2$O), 4.15 (d, 1 H, J = 7.5 Hz, H-1), 4.13 (m, 1 H, H-6a'), 4.05 (m, 2 H, H-6a and H-5), 3.88 (m, 1 H, O*CH*(CH$_2$)$_7$), 3.75 (d, 1 H, J = 5.5, 10.0 Hz, H-6b'), 3.65 (dd, 1 H, J = 6.5, 10.0 Hz, H-6b), 3.66 (s, 3 H, OMe), 3.60 (bs, 1 H, H-4), 3.44-3.32 (m, 2 H, H-5 and O*CH*(CH$_2$)$_7$), 3.18 (dd, 1 H, J = 2.5, 10.0 Hz, H-3), 3.10 (dd, 1 H, J = 7.5, 10.0 Hz, H-2), 2.30 (t, 2 H, *CH*$_2$CO$_2$Me), 2.20, 2.05, and 1.90 (3 s, 9 H, 3 x OAc).

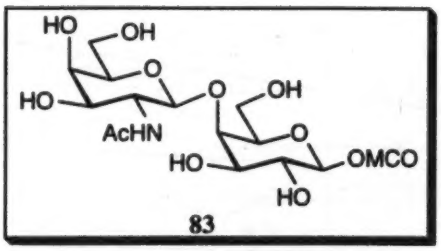
8-Methoxycarbonyloctyl 4-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl)-2,3,6-tri-O-benzyl-β-D-galactopyranoside (**82**).



82

A solution of compound **81** (7.9 g, 6.75 mmol) and hydrazine acetate (6.2 g, 67.5 mmol) in MeOH (240 mL) was refluxed for 5 h. To the refluxing solution, more hydrazine acetate (6 g) was added. The solution was kept refluxing overnight and then concentrated and co-evaporated with toluene (2 x 100 mL). A solution of the resulting residue in Ac_2O-pyridine (1:1, 170 mL) was stirred for 4 h at rt, diluted with CH_2Cl_2 (300 mL), washed with ice-HCl (5%, 2 x 100 mL) and then with H_2O, dried with $MgSO_4$, filtered, and concentrated. The residue was passed through a silica gel column (hexane-EtOAc, 1:1 to 1:2) to give **82** (5.5 g, 85%). ^{1}H NMR (CD_3OD): δ = 7.45-7.25 (m, 15 H, aromatics), 5.13 (dd, 1 H, J = 11.5, 3.5 Hz, H-3'), 5.31 (d, 1 H, J = 3.5, H-4'), 4.88 (d, 1 H, J = 8.5 Hz, H-1'), 4.92 and 4.67 (2 d, 2 H, J = 11.5 Hz, Ph*CH*$_2$O), 4.74 and 4.70 (2 d, 2 H, J = 11.5 Hz, Ph*CH*$_2$O), 4.55 (s, 2 H, Ph*CH*$_2$O), 4.38 (d, 1 H, J = 7.5 Hz, H-1), 4.12 (dd, 1 H, J = 7.5, 11.5 Hz, H-2), 4.05-3.85 (m, 5 H, H-4, H-5, 2 x H-6', and O*CH*$(CH_2)_7$), 3.52-3.50 (m, 5 H, H-5', 2 x H-6, H-2', and O*CH*$(CH_2)_7$), 3.66 (s, 3 H, OMe), 2.30 (t, 2 H, *CH*$_2$CO$_2$Me), 2.15, 1.98, and 1.90 (3 s, 9 H, 3 x OAc).

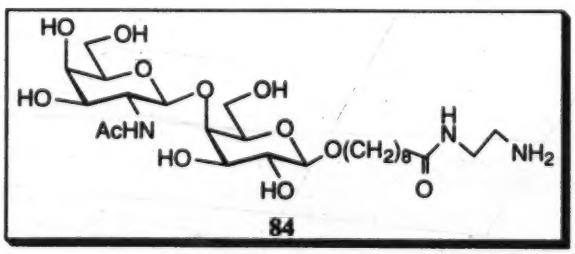
8-Methoxycarbonyloctyl 4-O-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-β-D-galactopyranoside (**83**).



83

To a suspension of compound **82** (5 g) in dry MeOH (200 mL), NaOMe (540 mg) was added. The mixture was stirred overnight at rt and then neutralized with Amberlite IR-120 [H^+] and filtered. The resin was washed with MeOH and the combined MeOH solution was concentrated to about 300 mL volume. To the methanol solution, $Pd(OH)_2/C$ (20%, 1.2 g) was added. The mixture was stirred under H_2 overnight and then filtered through a Celite pad and then concentrated. The residue was applied to an Iatrabeads column (CH_2Cl_2-MeOH, 8:1, 4:1 to 3:1). The collection was evaporated to white solid, which was purified again by C-18 column adsorption (H_2O, H_2O-MeOH 1:1, and MeOH), after lyophilized, to give white powder **83** (90%). ^1H NMR (D_2O): δ = 4.64 (d, 1 H, J = 8.5 Hz, H-1'), 4.18 (d, 1 H, J = 7.9 Hz, H-1), 4.02 (d, 1 H, J = 3.0 Hz, H-4), 3.91-3.47 (m, 12 H, H-2', H-3', H-4', H-5', 2 x H-6', H-3, H-5, 2 x H-6, and $OCH_2(CH_2)_7COOMe$), 3.43 (dd, 1 H, J = 7.9, 10.0 Hz, H-2), 3.65 (s, 3 H, OMe), 2.31 (t, 2 H, J = 7.5 Hz, CH_2COMe), 2.03 (s, 3 H, NAc), 1.60 and 1.35 (b, 12 H, $OCH_2(CH_2)_6CH_2COOMe$). ^{13}C NMR (D_2O): δ = 178.7, 175.8, 103.6, 103.4, 76.7, 75.7, 74.9, 73.7, 71.8, 71.7, 71.3, 68.7, 61.9, 61.2, 53.6, 52.9, 34.6, 29.7, 29.07, 29.01, 28.96, 25.8, 25.1, and 23.2.

8-(2-aminoethyl)carboxamidooctyl 4-O-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-β-D-galactopyranoside (**84**).

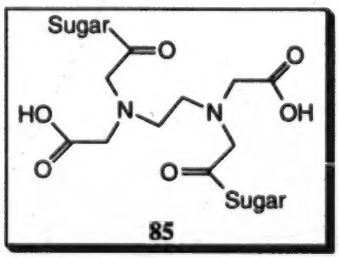


84

A solution of disaccharide **83** (350 mg) in neat anhydrous ethylenediamine (refluxed and distilled from sodium, 100 mL) was heated at 70 °C with stirring

for 2 d and then concentrated and co-concentrated with toluene (3 x 50 mL) to remove the excess of ethylenediamine. The resulting residue was chromatographed on an Iatrobeads column (3:1, MeOH-CH_2Cl_2 containing 1% of aq NH_4OH) and then isolated as described for **83**. The solution of product was passed through a Millipore filter and the filtrate was lyophilized to provide a white powder **84** (359 mg, 95%; $[\alpha]_D$ = -7.1°, *c* = 0.4, in MeOH; R_f = 0.23, MeOH-CH_2Cl_2, 3:1 containing 1% of aq. NH_4OH). ^{1}H NMR (CD_3OD): δ = 4.63 (d, 1 H, J = 8.5 Hz, H-1'), 4.18 (d, 1 H, J = 7.9 Hz, H-1), 4.02 (d, 1 H, J = 3.0 Hz, H-4), 3.75 (d, 1 H, J = 3.0 Hz, H-4'), 3.91-3.77 and 3.74-3.46 (m, 11 H, H-2', H-3', H-5', 2 x H-6', H-3, H-5, 2 x H-6, and O*CH*$_2$(CH_2)$_7$CONH), 3.44 (dd, 1 H, J = 7.9 Hz, 10.0 Hz, H-2), 3.26 (t, 2 H, J = 6.5 Hz, CONH*CH*$_2$), 2.75 (t, 2 H, J = 6.5 Hz, *CH*$_2$ NH_2), 2.20 (t, 2 H, J = 7.5 Hz, *CH*$_2$CONH), 2.03 (s, 3 H, NAc), 1.60 and 1.35 (b, 12 H, OCH$_2$(*CH*$_2$)$_6$CH$_2$CONH). ^{13}C NMR (D_2O): δ = 178.8, 175.9, 103.6, 103.4, 76.7, 75.7, 74.9, 73.6, 71.8, 71.7, 71.3, 68.7, 61.9, 61.2, 53.5, 40.3, 40.2, 36.6, 29.6, 29.1, 29.0, 28.9, 26.0, 25.7, and 23.2. FAB-MS ($C_{25}H_{47}N_3O_{12}$, MW: 581): *m/z* 582 $[M+H]^+$ and 604 $[M+Na]^+$.

N,N'-di-{8-[4-O-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-β-D-galactopyranosyloxy]octylcarbonylaminoethyl}ethylenediaminediacetamido-N,N'-diacetic acid (**85**).

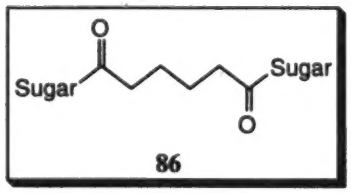


85

To a solution of **84** (5 mg, 8.6 μmol) in dry pyridine (0.6 mL), ethylenediaminetetraacetic dianhydride (0.85 mg, 3.3 μmol) was added at rt. TLC indicated the absence of the EDTA dianhydride (R_f = 0.35, MeOH-CH_2Cl_2-H_2O, 4:2:1) after 4 h. The solution was concentrated and then co-evaporated with toluene (3 x 1 mL) to remove pyridine. The residue was applied to an Iatrobeads column (MeOH-CH_2Cl_2-H_2O, 4:2:1) and then to a C-

18 Sep-Pak cartridge to give product **85** (4.7 mg, 99%; $[\alpha]_D$ = -5.3°, *c* = 0.36, in MeOH). ^{1}H NMR (D_2O): δ = 4.64 (d, 2 H, J = 8.5 Hz, 2 x H-1'), 4.38 (d, 2 H, J = 7.9 Hz, 2 x H-1), 4.08 (d, 2 H, J = 3.0 Hz, 2 x H-4), 4.0-3.0 (m, 46 H, 2 x H-2', 2 x H-3', 2 x H-4', 2 x H-5', 4 x H-6', 2 x H-3, 2 x H-2, 2 x H-5, 4 x H-6, 2 x O*CH*$_2$(CH$_2$)$_7$CONH, 12 H for EDTA, and 2 x CONH*CH*$_2$*CH*$_2$NH), 2.23 (t, 4 H, J = 7.5 Hz, 2 x O(CH$_2$)$_7$*CH*$_2$CONH), 2.06 (s, 6 H, 2 x NAc), 1.60 and 1.35 (b, 24 H, 2 x OCH$_2$(*CH*$_2$)$_6$CH$_2$CONH). ^{13}C NMR (D_2O): δ = 176.7, 175.2, 174.0, 104.9, 104.3, 78.4, 76.9, 75.5, 74.9, 74.7, 72.8, 70.8, 69.6, 62.7, 61.4, 60.9, 59.5, 55.6, 55.4, 54.2, 49.9, 49.6, 49.3, 49.0, 48.7, 48.4, 48.1, 40.0, 39.9, 37.1, 30.7, 30.3, 30.2, 26.9, 26.8, and 23.1. FAB-MS ($C_{60}H_{106}N_8O_{30}$, MW: 1419): *m/z* 1442 $[M+Na]^+$, 1458 $[M+K]^+$, 1464 $[M-H+2Na]^+$ and 1480 $[M-H+2K]^+$.

N,N'-di-{8-[4-O-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-β-D-galactopyranosyloxy]-octylcarbonylaminoethyl}adipamide (**86**).

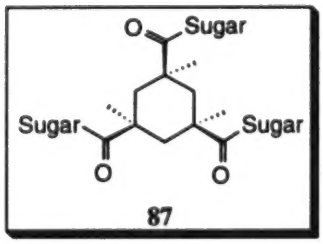


86

To a solution of **84** (5 mg, 8.6 μmol) and adipic acid (0.5 mg, 3.4 μmol) in dry MeOH (2 mL), 1-[3-(dimethylamino)propyl]ethylcarbodiimide hydrochloride (EDC) (6.5 mg, 33.9 μmol), 1-hydroxy-benzotriazole (HOBt) (4.5 mg, μmol) and Et_3N (6.6 μL, 47.6 μmol) were added at rt. After stirred 10 h, the solution was concentrated. Column chromatography (Iatrobeads, Pr^iOH-MeOH-NH_4OH, 6:6:5), followed by filtration with a Millipore filter and lyophilization gave a white powder **86** (3.5 mg, 81%; R_f = 0.08, Pr^iOH-MeOH-NH_4OH, 3:3:2; $[\alpha]_D$ = -8.6°, *c* = 0.2, in MeOH). ^{1}H NMR (D_2O): δ = 4.63 (d, 2 H, J = 8.5 Hz, 2 x H-1'), 4.38 (d, 2 H, J = 8.0 Hz, 2 x H-1), 4.08 (d, 2 H, J = 2.6 Hz, 2 x H-4), 3.95-3.30 (m, 34 H, 2 x H-2', 2 x H-3', 2 x H-4', 2 x H-5', 4 x H-6', 2 x H-2, 2 x H-3, 2 x H-5, 4 x H-6, 2 x

O*CH*$_2$(CH$_2$)$_7$CONH, and 2 x CONH*CH*$_2$*CH*$_2$NH), 2.26-2.18 (m, 8xH, 2 x O(CH$_2$)$_7$*CH*$_2$CONH and CONH*CH*$_2$(CH$_2$)$_2$*CH*$_2$CONH), 2.06 (s, 6 H, 2 x Ac), 1.58 and 1.31 (b, 28 H, CONHCH$_2$(*CH*$_2$)$_2$CH$_2$CONH and 2 x OCH$_2$(*CH*$_2$)$_6$CH$_2$CONH). ^{13}C NMR (D_2O): δ = 178.3, 177.5, 175.8, 103.6, 103.4, 76.7, 75.7, 74.9, 73.7, 71.8, 71.7, 71.3, 68.7, 61.9, 61.2, 53.6, 39.5, 39.3, 36.7, 36.3, 29.6, 29.2, 29.0, 26.2, 25.8, 25.6, and 23.3. FAB-MS ($C_{56}H_{100}N_6O_{26}$, MW: 1273): *m/z* 1274 $[M+H]^+$, 1296 $[M+Na]^+$ and 1312 $[M+K]^+$.

N,N',N"-tri-{8-[4-O-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-β-D-galactopyranosyloxy]octylcarbonylaminoethyl}-1,3,5-trimethyl-1,3,5-cyclohexanetriamide (**87**).

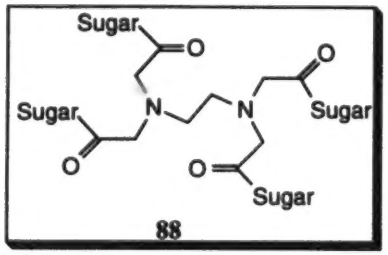


87

N,N'-Diisopropylcarbodiimide (DIC) (2.9 μL, 18.6 μmol) and HOBt (1.45 mg, 10.2 μmol) were added to a stirred solution of **84** (5.9 mg, 10.2 μmol) and Kemp's triacid (0.8 mg, 3.1 μmol) in dry DMF (0.2 mL) at 0 °C. The mixture was warmed to rt, stirred for 2 d and then added more DIC (3.0 μL). The solution was heated at 50 °C overnight and concentrated in vacuo. The residue was applied to an Iatrobeads column ($CHCl_3$-MeOH-H_2O, 5:4:1) and then a C-18 Sep-Pak cartridge. The main product was concentrated, filtered with a Millipore filter and lyophilized to give product **87** (3 mg, 50%; R_f = 0.35, $CHCl_3$-MeOH-H_2O, 5:4:1; $[\alpha]_D$ = -7.0°, *c* = 0.27, in MeOH). ^{1}H NMR (CD_3OD): δ = 4.63 (d, 3 H, J = 8.5 Hz, 3 x H-1'), 4.18 (d, 3 H, J = 8.0 Hz, 3 x H-1), 4.02 (d, 3 H, J = 2.8 Hz, 3 x H-4), 3.95-3.20 (m, 51 H, 3 x H-2', 3 x H-3', 3 x H-4', 3 x H-5', 6 x H-6', 3 x H-2, 3 x H-3, 3 x H-5, 6 x H-6, 3 x O*CH*$_2$(CH$_2$)$_7$CONH, and 3 x CONH*CH*$_2$*CH*$_2$NH), 2.74 and 1.16 (d, 3 H for each, J = 15 Hz, 3 x CH_2 on Kemp's triamide ring), 2.20 (t, 6 H, J = 7.5

Hz, 3 x $O(CH_2)_7CH_2CONH$), 2.02 (s, 9 x H, 3 x NAc), 1.60 and 1.33 (b, 36 H, 3 x $OCH_2(CH_2)_6CH_2CONH$), 1.22 (s, 9 H, 3 x Me on Kemp's triamide ring). ^{13}C NMR (CD_3OD): δ = 179.9, 176.5, 175.2, 104.9, 104.4, 78.5, 76.9, 75.5, 75.0, 74.8, 72.8, 70.9, 69.6, 62.7, 61.4, 55.6, 43.7, 43.4, 40.8, 39.6, 37.3, 33.5, 30.8, 30.4, 30.3, 27.04, 26.97, and 23.1. FAB-MS ($C_{87}H_{153}N_9O_{39}$, MW: 1948): *m/z* 1971 $[M+Na]^+$.

N,N,N',N'-tetra-{8-[4-O-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-β-D-galactopyranosyloxy]octylcarbonylaminoethyl}ethylenediaminetetraacetamide (**88**).

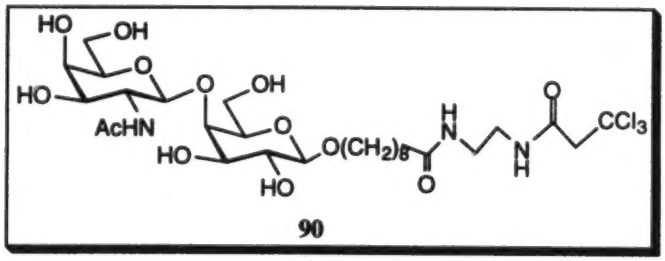


88

To a solution of **84** (10 mg, 17.2 μmol) and ethylenediaminetetraacetic acid (EDTA) (1.2 mg, 4.1 μmol) in dry DMF (1 mL) was added 1,3-dicyclohexylcarbodiimide (DCC) (8.5 mg, 41 μmol) and HOBt (2.8 mg, 17.2 μmol) at rt. The mixture was stirred for 4 d and then concentrated at 30 °C in vacuo, The resulting residue was purified with an Iatrobeads column (2 g, MeOH-CH_2Cl_2-H_2O-NH_4OH, 9:6:3:1) and then with a C-18 Sep-Pak cartridge. The fraction was concentrated, filtered with a Millipore filter and lyophilized to give a white powder **88** (4 mg, 38%, 30% of **84** recovered; R_f = 0.1, MeOH-CH_2Cl_2-H_2O-NH_4OH, 9:6:3:1; $[\alpha]_D$ = -4.4°, *c* = 0.27, in MeOH). ^1H NMR (D_2O): δ = 4.65 (d, 4 H, J = 8.4 Hz, 4 x H-1'), 4.38 (d, 4 H,J = 7.9 Hz, 4 x H-1), 4.09 (d, 4 H, J = 2.8 Hz, 4 x H-4), 3.96-3.60 (m, 48 H, 4 x H-2', 4 x H-3', 4 x H-4', 4 x H-5', 8 x H-6', 4 x H-3, 4 x H-5, 8 x H-6, and 4 x $OCH_2(CH_2)_7CO$), 3.39 (dd, 4 H, J = 7.9, 10.0 Hz, 4 x H-2), 3.36 (s, 16 H, 4 x $CONHCH_2CH_2NHCO$), 3.27 (s, 8 H, 4 x $NHCH_2CONH$), 2.70 (s, 4 H, NCH_2CH_2N), 2.23 (t, 8 H, J = 7.5 Hz, 4 x $O(CH_2)_7CH_2CO$), 2.07 (s, 12 H, 4 x NAc), 1.60 and 1.31 (m, 48 H, 4 x $OCH_2(CH_2)_6CH_2CO$). ^{13}C NMR (D_2O): δ = 178.1, 175.8, 174.2, 103.6, 103.4, 76.7,

75.7, 74.9, 73.7, 71.8, 71.7, 71.3, 68.7, 61.9, 61.2, 59.2, 53.6, 39.7, 36.8, 30.7, 29.6, 29.2, 29.1, 26.3, 25.9, and 23.3. FAB-MS ($C_{110}H_{196}N_{14}O_{52}$, MW: 2545): 2568 $[M+Na]^+$.

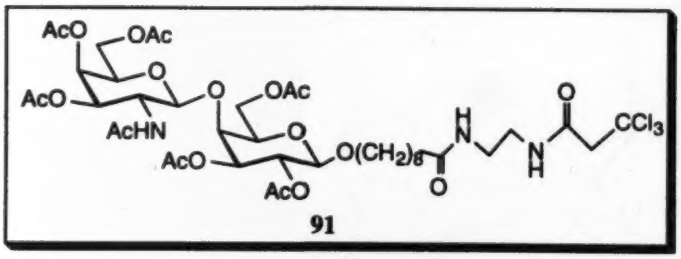
8-[2-(2,2,2-trichloroethoxycarbonyl)aminoethyleneaminocarbonyl]octyl 4-O-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-β-D-galactopyranoside (**90**).



90

Compound **84** (20 mg, 34.4 μmol) was dissolved in aq $NaHCO_3$ (1N, 1 mL) and 2,2,2-trichloroethylchloroformate (14 μL, 103.3 μmol) was added at rt. The mixture was stirred for 30 min, concentrated and co-evaporated with toluene (3 x 1 mL). The residue was applied to an Iatrobeads column (Pr^iOH-MeOH-NH_4OH, 2:2:1) to give Troc-derivative **90** (25 mg, 98%; R_f = 0.53, Pr^iOH-MeOH-NH_4OH, 3:3:2; $[\alpha]_D$ = -6.9°, *c* = 0.19, in MeOH). ^1H NMR (CD_3OD): δ = 4.74 (s, 2 H, OCH_2Cl_3), 4.63 (d, 1 H, J = 8.5 Hz, H-1'), 4.18 (d, 1 H, J = 7.9 Hz, H-1), 4.01 (d, 1 H, J = 3.0 Hz, H-4), 3.90-3.45 (m, 12 H, H-2', H-3', H-4', H-5', 2 x H-6', H-3, H-5, 2 x H-6, and $OCH_2(CH_2)_7CO$), 3.42 (dd, 1 H, J = 7.9 Hz, 10.0 Hz, H-2), 3.26 (m, 4 H, $CONHCH_2CH_2NHCO$), 2.06 (t, 2 H, J = 7.5 Hz, $O(CH_2)_7CH_2CO$), 2.02 (s, 3 H, NAc), 1.60 and 1.30 (bs, 12 H, $OCH_2(CH_2)_6CH_2CO$).

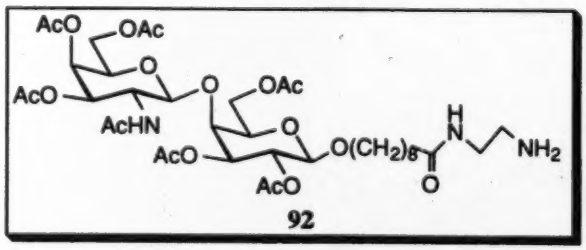
8-[2-(2,2,2-trichloroethoxycarbonyl)aminoethyleneaminocarbonyl]octyl 4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-2,3,6-tri-O-acetyl-β-D-galactopyranoside (**91**).



91

A solution of compound **90** (25 mg, 33.7 μmol) in Ac_2O-pyridine (1:1, 1 mL) was stirred overnight at rt and then concentrated. The resulting residue was chromatographed (SiO_2, CH_2Cl_2-MeOH, 20:1) to give **91** (30 mg, 88%; R_f = 0.68, CH_2Cl_2-MeOH 10:1; $[\alpha]_D$ = -12.0°, *c* = 0.19, in $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 6.40 (bs, 1 H, CO*NH*CH_2), 6.02 (d, 1 H, J = 7.0 Hz, *NH*Ac), 5.91 (dd, 1 H, J = 11.5, 3.0 Hz, H-3'), 5.75 (bs, 1 H, CO*NH*CH_2), 5.38 (d, 1 H, J = 3.0 Hz, H-4'), 5.24 (dd, 1 H, J = 7.9, 10.5 Hz, H-2), 5.12 (d, 1 H, J = 8.2 Hz, H-1'), 4.95 (dd, 1 H, J = 10.5, 2.5 Hz, H-3), 4.55 (s, 2 H, O*CH*$_2$$CCl_3$), 4.42 (d, 1 H, J = 7.9 Hz, H-1), 4.28 (m, 2 H, 2 x H-6), 4.14 (d, 1 H, J = 2.5 Hz, H-4), 4.05 (d, 2 H, J = 6.5 Hz, 2 x H-6'), 3.92 (d, 1 H, J = 6.5 Hz, H-5'), 3.88 (m, 1 H, O*CH*$(CH_2)_7$CO), 3.74 (t, 1 H, J = 6.0 Hz, H-5), 3.50-3.33 (m, 5 H, O*CH*$(CH_2)_7$CO and CONH*CH*$_2$*CH*$_2$NHCO), 2.18 (t, 2 H, J = 7.0 Hz, O$(CH_2)_7$*CH*$_2$CO), 2.14-1.97 (7 s, 21 H, 7 x Ac), 1.60 and 1.30 (bs, 12 H, OCH_2(*CH*$_2$)$_6$$CH_2$CO).

8-(2-aminoethyleneamonocarbonyl)octyl 4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-2,3,6-tri-O-acetyl-β-D-galactopyranoside (**92**).

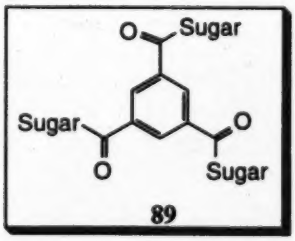


92

Zinc dust (30 mg) was added to a solution of Troc-derivative **91** (29 mg, 28.7 μmol) in HOAc (0.5 mL) at rt. The suspension was stirred overnight and then filtered through a Celite pad. The Celite pad and zinc dust were washed with

CH_2Cl_2. The combined CH_2Cl_2 solution was partitioned with water and the aqueous layer was washed with CH_2Cl_2. The organic solution was dried, evaporated to a syrup and dissolved in CH_2Cl_2 for chromatography (SiO_2, CH_2Cl_2-MeOH, 8:1). Evaporation of the main fraction gave product **92** (20 mg, 84%; R_f = 0.13, CH_2Cl_2-MeOH, 8:1; $[\alpha]_D$ = -15.6°, c = 0.5, in $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 6.28 (d, 1 H, J = 6.8 Hz, *NH*Ac), 6.12 (bs, 1 H, CO*NH*CH_2CH_2), 5.95 (dd, 1 H, J = 11.5, 3.3 Hz, H-3'), 5.40 (d, 1 H J = 3.0 Hz, H-4'), 5.24 (dd, 1 H, J = 8.0, 10.5 Hz, H-2), 5.14 (d, 1 H, J = 8.2 Hz, H-1'), 4.94 (dd, 1 H, J = 10.5, 2.5 Hz, H-3), 4.42 (d, 1 H, J = 8.0 Hz, H-1), 4.29 (d, 2 H, J = 6.0 Hz, 2 x H-6), 4.13 (d, 1 H, J = 2.5 Hz, H-4), 4.05 (d, 2 H, J = 6.5 Hz, 2 x H-6'), 3.92 (d, 1 H, J = 6.5 Hz, H-5'), 3.88 (m, 1 H, O*CH*$(CH_2)_7$CO), 3.74 (t, 1 H, J = 6.0 Hz, H-5), 3.47 (m, 1 H, O*CH*$(CH_2)_7$CO), 3.34 (m, 3 H, H-2' and CONH*CH*$_2$ CH_2NH_2), 2.85 (bt, 2 H, $CONHCH_2CH_2NH_2$), 2.19 (t, 2 H, J = 7.5 Hz, $O(CH_2)_7CH_2CO$), 2.15-1.97 (7 s, 21 H, 7 x Ac), 1.60 and 1.30 (bs, 12 H, $OCH_2(CH_2)_6CH_2CO$).

N,N',N"-tri-{8-[4-O-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-β-D-galactopyranosyl-oxy]octylcarbonylaminoethyl}-1,3,5-benzenetriamide (**89**).



89

To a solution of compound **92** (8.3 mg, 9.9 μmol) and Et_3N (1.4 μL, 9.9 μmol) in dry CH_2Cl_2 (0.5 mL), 1,3,5-benzentricarbonyltrichloride (0.88 mg, 3 μmol) was added at rt. The resulting solution was stirred for 10 min and then concentrated. The residue was purified with chromatography (SiO_2, CH_2Cl_2-MeOH, 15:1) to give acetylated **89** (8.7 mg, 99%; R_f = 0.56, CH_2Cl_2-MeOH, 8:1; $[\alpha]_D$ = -12.8°, c = 0.3, in $CHCl_3$). Selected NMR data: ^{1}H NMR ($CDCl_3$): δ = 8.65 (s, 3 H, aromatic). ^{13}C NMR ($CDCl_3$): δ = 193.9, 174.5, 171.6, 171.0, 170.7, 170.5, 170.2, 170.0, and 169.7 (9 x Ac), 134.7 and 129.0 (aromatic). To a solution of

acetylated **89** (8.0 mg) in dry MeOH (5 mL), NaOMe (13.5 mg) was added at rt. After three hours, the solution was neutralized with Dowex-50W (H^+) exchange resin, filtered and concentrated. The resulting residue was chromatographed with an Iatrobeads column (Pr^iOH-MeOH-H_2O, 3:3:1) and then with a C-18 Sep-Pak cartridge to give a white powder **89** (5.4 mg, 94%; R_f = 0.28, Pr^iOH-MeOH-H_2O, 3:3:1; $[\alpha]_D$ = -5.3°, c = 0.3, in MeOH). ^1H NMR (D_2O): δ = 8.35 (s, 3 H, aromatic), 4.64 (d, 3 H, J = 8.5 Hz, 3 x H-1'), 4.34 (d, 3 H, J = 7.9 Hz, 3 x H-1), 4.08 (d, 3 H, J = 2.8 Hz, 3 x H-4), 3.94-3.48 (m, 48 H, 3 x H-2', 3 x H-3', 3 x H-4', 3 x H-5', 6 x H-6', 3 x H-3, 3 x H-5, 6 x H-6, 3 x O*CH*$_2$(CH$_2$)$_7$CONH, and 3 x CONH*CH*$_2$*CH*$_2$NH), 3.38 (dd, 3 H, J = 7.9, 10.0 Hz, 3 x H-2), 2.22 (t, 6 H, J = 7.0 Hz, 3 x O(CH$_2$)$_7$*CH*$_2$CONH), 2.07 (s, 9 H, 3 x NAc), 1.48 and 1.11 (b, 18 H, 3 x OCH$_2$*(CH*$_2$*)*$_6$CH$_2$CONH). ^{13}C NMR (D_2O): δ = 178.4, 175.8, 169.0, 135.6, 129.9, 103.6, 103.4, 76.8, 75.7, 74.9, 73.7, 71.9, 71.7, 71.2, 68.7, 61.9, 61.1, 53.6, 40.4, 39.3, 36.7, 29.6, 29.2, 29.1, 19.0, 26.2, 25.8, and 23.3. FAB-MS ($C_{84}H_{141}N_9O_{39}$, MW: 1899): *m/z* 1922 $[M+Na]^+$ and 1939 $[M+K]^+$.

PART II

Studies on 2-Amino-2-deoxy Glycosyl Donors

Chapter 4

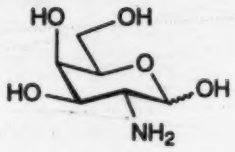
Introduction

4.1. Amino Sugars in Nature.

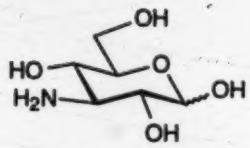
An amino sugar is a carbohydrate derivative where one (or more) of the hydroxyl groups attached to the carbon backbone of a sugar molecule is replaced by a free or substituted amino group. Nitrogen normally adopts a valence state different from that of oxygen and sulfur. Its introduction into a sugar therefore constitutes a functional group replacement rather than the electronic homologation that relates the thio sugars to their corresponding oxygenated counterparts [102]. The accepted nomenclature is based on replacement terminology, for example, 2-amino-2-deoxy-α/β-D-galactopyranose (**96**), 3-amino-3-deoxy-α/β-D-glucopyranose (**97**), and 2,4-diamino-2,4,6-trideoxy-α/β-D-glucopyranose (**98**) (Fig. 4.1). Part II of this thesis focuses on the occurrence and synthesis of 2-amino sugars.

4.1.1. 2-Amino-2-deoxy Sugars.

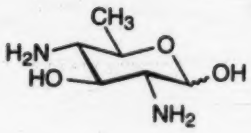
2-Amino-2-deoxy sugars are the most widely distributed class of amino-sugar in nature. 2-Amino-2-deoxy-D-glucose (D-glucosamine or chitosamine) was the first amino sugar found in nature in 1876 [103]. It is one of the most abundant monosaccharides and occurs as a major constituent in the hard shells of crustaceans and other arthropods, in



96



97



98

Fig. 4.1: Structures of some amino sugars.

many fungi and in higher animals. Glucosamine was also identified as a constituent of glycosylphosphatidylinositols which are membrane anchors for cell-surface glycoproteins [113]. Chitin is the most abundant of those polysaccharides that contain 2-amino-2-deoxy-D-glucose [114]. It is found in most fungi, mycelial yeasts, green algae and several species of brown and red algae [114]. It is also widespread in the animal kingdom, occurring in the form of sheets as in the cuticles of arthropods, annelids and molluscs, or in the form of well-oriented fibers as in the mandibular tendon of lobster [114].

2-Amino-2-deoxy-D-galactose (D-galactosamine or chondrosamine) is the second amino sugar found in nature in 1914 [104]. It is a constituent of the antibiotic racemomycin and of numerous bacterial polysaccharides. There are also other 2-amino-2-deoxy sugars in nature, such as 2-amino-2,6-dideoxy-D- and L-galactose [105], which occur as a constituent of antigenic Type V pneumococcal capsular polysaccharide; 2-amino-2,6-dideoxy-D-glucose, which was isolated from a bacterial polysaccharide, and 2-deoxy-2-methylamino-L-glucose (the third amino sugar to be found in Nature and the first one in an economically significant product), which is a constituent of the antibiotic streptomycin [106]; 2-amino-2-deoxy-D-gulose, which was recognized as a component residue of the antibiotics streptothricin and streptolin, and 2-deoxy-2-methylamino-L-gulose, which was identified as a constituent of streptothricin analogs LL-AC541 and LL-AB644 [107]; 2-amino-2-deoxy-D-mannuronic acid, which was identified as a probable constituent of the cell-wall polysaccharide of *Micrococcus lysodeikticus*, and 2-amino-2,6-

dideoxy-L-mannose, which is a component of lipopolysaccharide in *Escherichia coli* U 41/14 [108]; 2-amino-2-deoxy-D-talose, which has been identified as a (probable) minor constituent of ovine and bovine cartilage, and 2-amino-2,6-dideoxy-D-talose, which occurs in Type V pneumococcal capsular polysaccharide [109]; 2-amino-2,3-dideoxy-D-ribohexose, which has been shown to be a constituent of the antibiotics lividomycin A and B [110], and 2-amino-2-deoxy-L-xylonic acid, which has been identified as a constituent of several antifungal agents [111].

4.1.2. Naturally Occurring *N*-acetylglucosamine.

2-Acetamido-2-deoxy-D-glucose (*N*-acetylglucosamine) and 2-acetamido-2-deoxy-D-galactose (*N*-acetylgalactosamine) are prominent constituents of glycoconjugates in nature. 2-Acetamido-2-deoxy-D-glucose is part of the core and of the side-chains in the glycan chains of *N*-glycoproteins. It is widely distributed in proteoglycans, in bacterial lipopolysaccharides and in the murein of bacterial cell-walls. GlcNAc is found in a variety of different glycosidic linkages (Table 4.1) [112], most of which are β.

Glycosidic linkage	Acceptor	Occurrence
β-(1→4)	GlcNAc	Chitobiose core structure of *N*-glycoproteins
β-(1→4)	MurAc	Part of murein of Gram-negative bacteria
β-(1→6)	GlcNAc	Disaccharide unit of lipid A (as in *Salmonella minnesota*)
β-(1→6)	GalNAc	Part of core structure of the *O*-glycoproteins
β-(1→3)	GalNAc	Part of core structure of the *O*-glycoproteins
β-(1→3)	Gal	*lacto*- and *neolacto*-series of glycosphingolipids
β-(1→6)	Gal	*lacto*- and *neolacto*-series of glycosphingolipids
β-(1→3)	Man	*artho*-series of glycosphingolipids
α-(1→6)	GlcA	Phosphoglycosphingolipids of tobacco leaves
β-(1→2)	Man	Phosphoglycosphingolipids

Table 4.1: Naturally occurring glycosidic linkages of *N*-acetylglucosamine.

4.1.3. *N*-Acetylgalactosamine-containing Glycosphingolipids.

2-Acetamido-2-deoxy-D-galactose is a constituent of the core structure of mucin-type oligosaccharides. The resulting *O*-glycoproteins constitute, along with the *N*-glycoproteins, a major class of glycoconjugates. In glycosphingolipids, *N*-acetylgalactosamine is mainly encountered in the *globo*, *isoglobo*, and *ganglio* series [112]. Some representative examples are shown in Table 4.2 [112].

Gala-Series
α-GalNAc-(1→3)-β-GalNAc-(1→3)-α-Gal-(1→4)-β-Gal-(1→O)-Cer

Globo-Series
β-GalNAc-(1→3)-α-Gal-(1→4)-β-Gal-(1→4)-β-Glc-(1→O)-Cer
α-Gal-(1→3)-β-GalNAc-(1→3)-α-Gal-(1→4)-β-Gal-(1→4)-β-Glc-(1→O)-Cer
β-Gal-(1→3)-β-GalNAc-(1→3)-α-Gal-(1→4)-β-Gal-(1→4)-β-Glc-(1→O)-Cer

Isoglobo-Series
β-GalNAc-(1→3)-α-Gal-(1→4)-β-Gal-(1→4)-β-Glc-(1→O)-Cer

Ganglio-Series
β-GalNAc-(1→4)-α-Gal-(1→4)-β-Glu-(1→O)-Cer
β-Gal-(1→3)-α-GalNAc-(1→4)-β-Gal-(1→4)-β-Glc-(1→O)-Cer

Lacto-Series
α-GalNAc-(1→3)-β-Gal-(1→3)-β-GlcNAc-(1→3)-β-Glc-(1→4)-β-Glc-(1→O)-Cer
2
↑
α-Fuc1

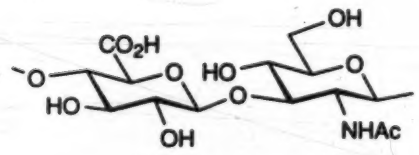
Arthro-Series
β-GalNAc-(1→4)-β-GlcNAc-(1→3)-β-Man-(1→4)-β-Glc-(1→O)-Cer
α-GalNAc-(1→4)-β-GalNAc-(1→4)-β-GlcNAc-(1→4)-β-Man-(1→4)-β-Glc-(1→O)-Cer

Phosphoglycosphingolipids
4-OMe-β-Gal-(1→3)-β-GalNAc-(1→3)-α-Fuc-(1→4)-β-GlcNAc-(1→2)-Man

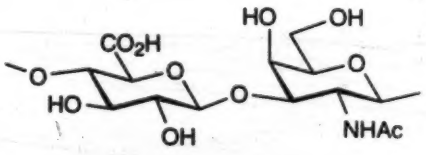
Table 4.2: Structures of *N*-acetylgalactosamine-containing glycosphingolipids.

4.1.4. Glycosaminoglycans.

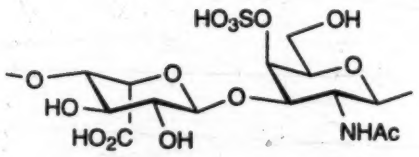
Proteoglycans and glycosaminoglycans are essential macromolecular components of mammalian bodies, occurring extensively in almost all mammalian tissues. They are therefore of prime importance in health and disease. Glycosaminoglycans generally are composed of *N*-acetylated or *N*-sulphated 2-amino-2-deoxy-D-glucose or 2-amino-2-deoxy-D-galactose. Eight glycosaminoglycans that have been identified are named in terms derived from hyaloid (vitreous), chondros (Greek for cartilage), derm (skin), hepar (Greek for liver) or keras (Greek for horn) (Fig. 4.2) [115, 116].



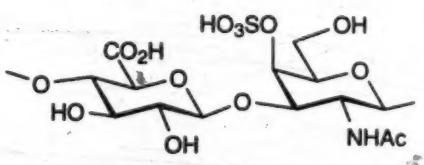
Hyaluronic acid



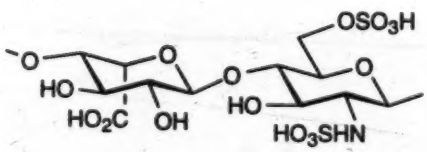
Chondroitin



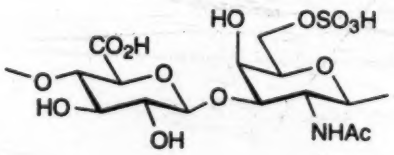
Dermatan 4-sulphate



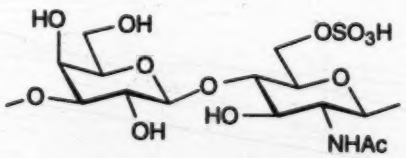
Chondroitin 4-sulphate



Heparin



Chondroitin 6-sulphate



Keratan sulphate

Fig. 4.2: Structures of some glycosaminoglycans.

In proteoglycans, the glycosaminoglycans are linked to the protein chain via a glycopeptide linkage. Research in the last decade has revealed that several types of glycopeptide linkage occur naturally. Representative examples are shown in Fig. 4.3a and Fig. 4.3b.

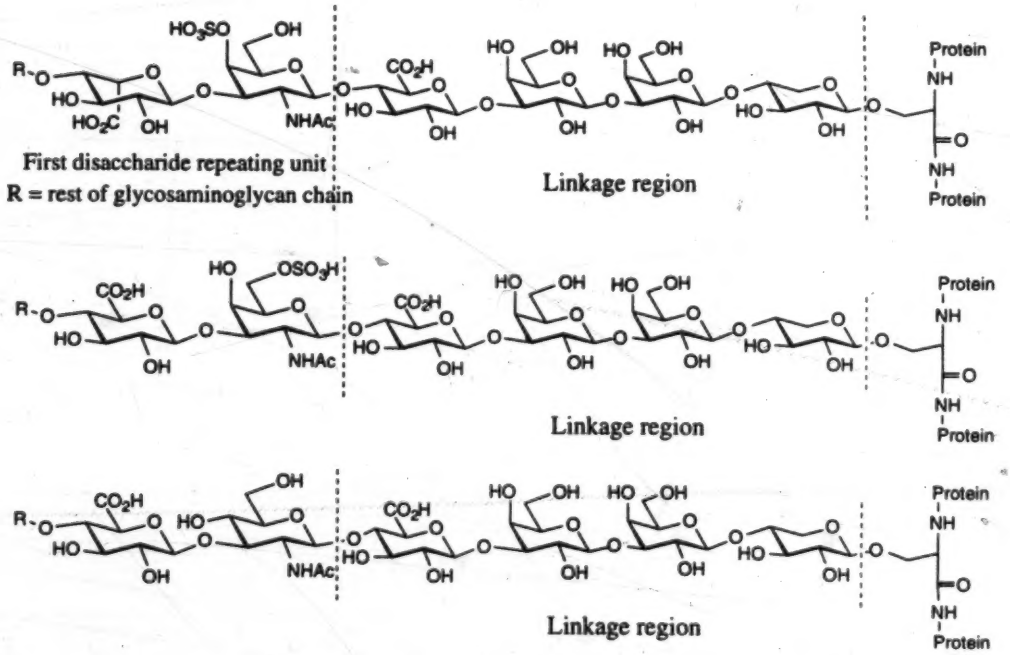


Fig. 4.3.a: Representive structures of glycosaminoglycans.

4.1.5. Aminoglycoside Antibiotics.

Many antibiotics also composed largely of carbohydrates have been obtained from micro-organisms and were termed aminoglycoside antibiotics because they contain several amino groups in their sugar moieties. Streptomycin was the first aminoglycoside antibiotic discovered in 1944. Many new aminoglycoside antibiotics have been discovered and their structures elucidated (Table 4.3) [117].

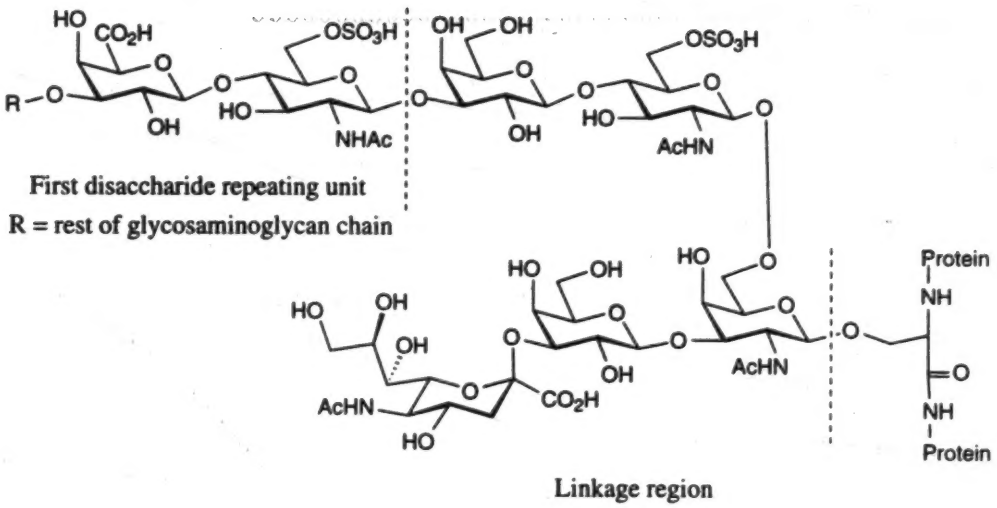


Fig. 4.3.b: Representive structure of glycosaminoglycan.

Year	*Antibiotics*	*Year*	*Antibiotics*
1944	Streptomycin	1967	α-D-Mannosyl-2-deoxy-
1947	Mannosidostreptomycin		α-D-glucoside
1949	Hydroxystreptomycin	1969	*N*-Demethylstreptomycin
	Neomycins		Hybrimycin
1956	Trehalosamine	1970	Ribostamycin
1957	Kanamycins		Sisomicin
1958	Hygromycin B		A-369-I
1959	Paromomycins	1971	Butirosins
1961	Spectinomycin		Lividomycins
1963	Bluensomycin		Tobramycin
	Gentamicins		Validamycin
1965	Kasugamycin	1973	apramycin
	Destomycin A		Bu-1709 E_1, E_2
			SS-56C

Table 4.3: The dates of discovery of some aminoglycoside Antibiotics.

4.2. Glycosylation Using 2-amino-2-deoxy-glycopyranosyl Donors.

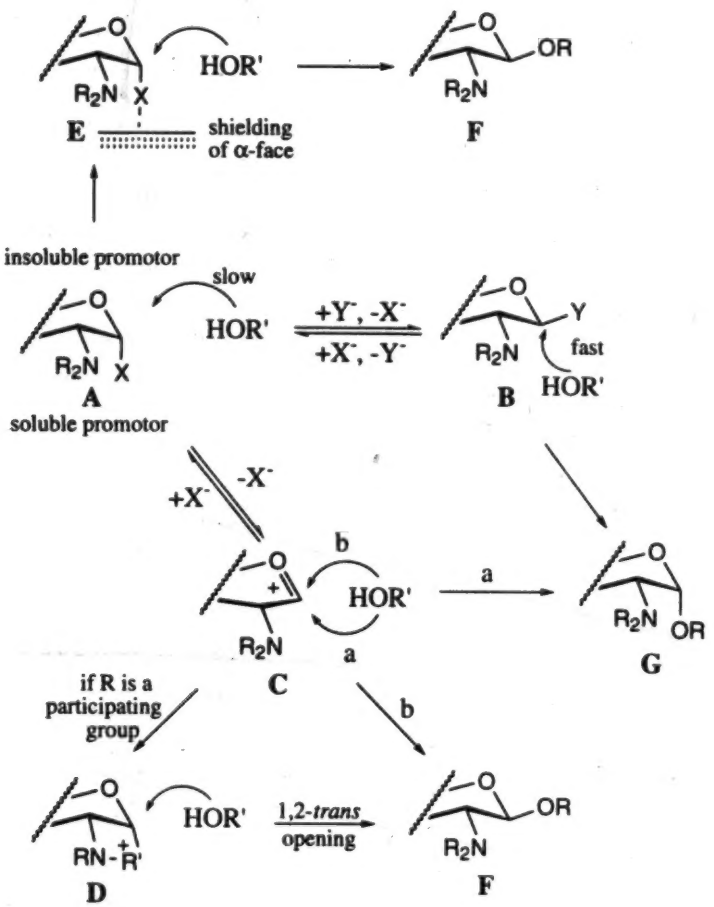
Many reviews and books devoted to oligosaccharide synthesis contain section devoted to the synthesis of 2-amino-2-deoxy glycosides [114, 117, 118-121]. The amino function has always been protected during the glycosylation reaction to avoid *N*-glycosylation because of its nucleophilicity. The choice of protecting group can provide control of stereoselectivity. The most commonly used methods for the construction of 1,2-*trans*-glycosidic linkages employ 2-amino sugar donors containing a participating group as the amino-protecting function. The ideal amino protecting group should be stable and impart sufficient reactivity, stereoselectivity and high yield in glycosylation reactions. Moreover, the protecting group should be readily removed under mild conditions and in high yield.

4.2.1. General Glycosylation Reaction Mechanism.

O-Glycosylation involves the creation of a carbon-oxygen bond via nucleophilic substitution. The glycosylation reaction is very complex, but some important features concerning it are now well understood. These are very useful for retrosynthetic analysis in planning the synthesis of a given oligosaccharide. Scheme 4.1 shows the general mechanism for glycosylation using 2-amino sugars [118].

The sugar containing the leaving group (X group) at the anomeric position is referred to as the glycosyl donor (A). The nucleophile, such as an alcohol or sugars with a free hydroxyl group, is referred to as the glycosyl acceptor. The reaction usually is effected in the presence of an activator called the reaction promotor. The role of the promotor is to assist the departure of the anomeric leaving group.

4.2.2. 1,2-*trans*-Glycosylations with 2-Amino-2-deoxy Sugars.



Scheme 4.1: General mechanism for the glycosylation of an 2-amino sugar.

Two general approaches are used to achieve 1,2-*trans*-glycosylation. The most widely used method involves a glycosyl donor containing a participating group as the amino protective function. This type of donor can form the intermediate **D** (Scheme 4.1) so the 1,2-*trans*-linked product **F** forms in a high stereoselectivity. Another method reported for the synthesis of the 1,2-*trans*-glycosides **F** uses the 1,2-*cis*-2-amino-2-deoxy-α-D-glycopyranosyl halides **A** (with a nonparticipating amino protecting group) and an insoluble promotor. The 1,2-*trans*-linkage **F** is formed since the insoluble promotor can

shield the α-face of the donor during the glycosylation reaction (intermediate **E**). This method is used mainly with 2-azido-2-deoxy donors and insoluble promotors. The stereoselectivities of this method are often lower than when donors contain C-2 participating groups.

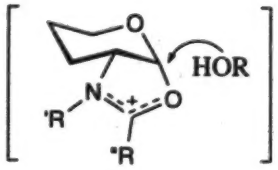


Fig. 4.5: Oxazolium intermediate in 1,2-*trans* glycosylation.

Many amino protecting groups have been developed for the 1,2-*trans*-glycosylation of 2-amino sugars. The *N*-phthalimido (NPhth) [121] is the most widely used. The *N*-acetamido (NAc) group has also been used [122] but the oxazolinium intermediate (Fig. 4.5, R' = H), presumed to be formed in the glycosylation reaction, is too stable making this donor unreactive. Generation of a free amine from either group usually requires strongly basic conditions that often cause partial product decomposition [123]. A number of alternative amino protecting groups have therefore been developed. These include *N*-4,5-dichlorophthaloyl (NDCP) [124], *N*-tetrachlorophthaloyl (NTCP) [125, 126], *N*-2,2,2-trichloroethoxycarbonyl (NTroc) [127], *N*-trichloroacetyl ($NCOCCl_3$) [128], *N*-dichloroacetyl ($NCOCHCl_2$) [129], *N*-monochloroacetyl ($NCOCH_2Cl$) [130], *N*-trifluoroacetyl ($NCOCF_3$) [131], *N*-sulfonyl (NSO_2Ph) [132], *N,N*-diacetyl (NAc_2) [133], *N*-acetyl-*N*-2,2,2-trichloroethoxycarbonyl (NAcTroc) [134], *N*-*p*-nitrobenzyloxycarbonyl (NPNZ) [135], *N*-2,5-dimethylpyrroyl (NDMP) [136], *N*-pent-4-enoyl [137], and *N*-dithiasuccinoyl (NDts) [138] groups. The structures and conditions for removal of these amino protecting groups are shown in Fig. 4.4. Presumably, all of the glycosylation reactions with these donors proceed via an oxazolinium intermediate (Fig. 4.5) with the

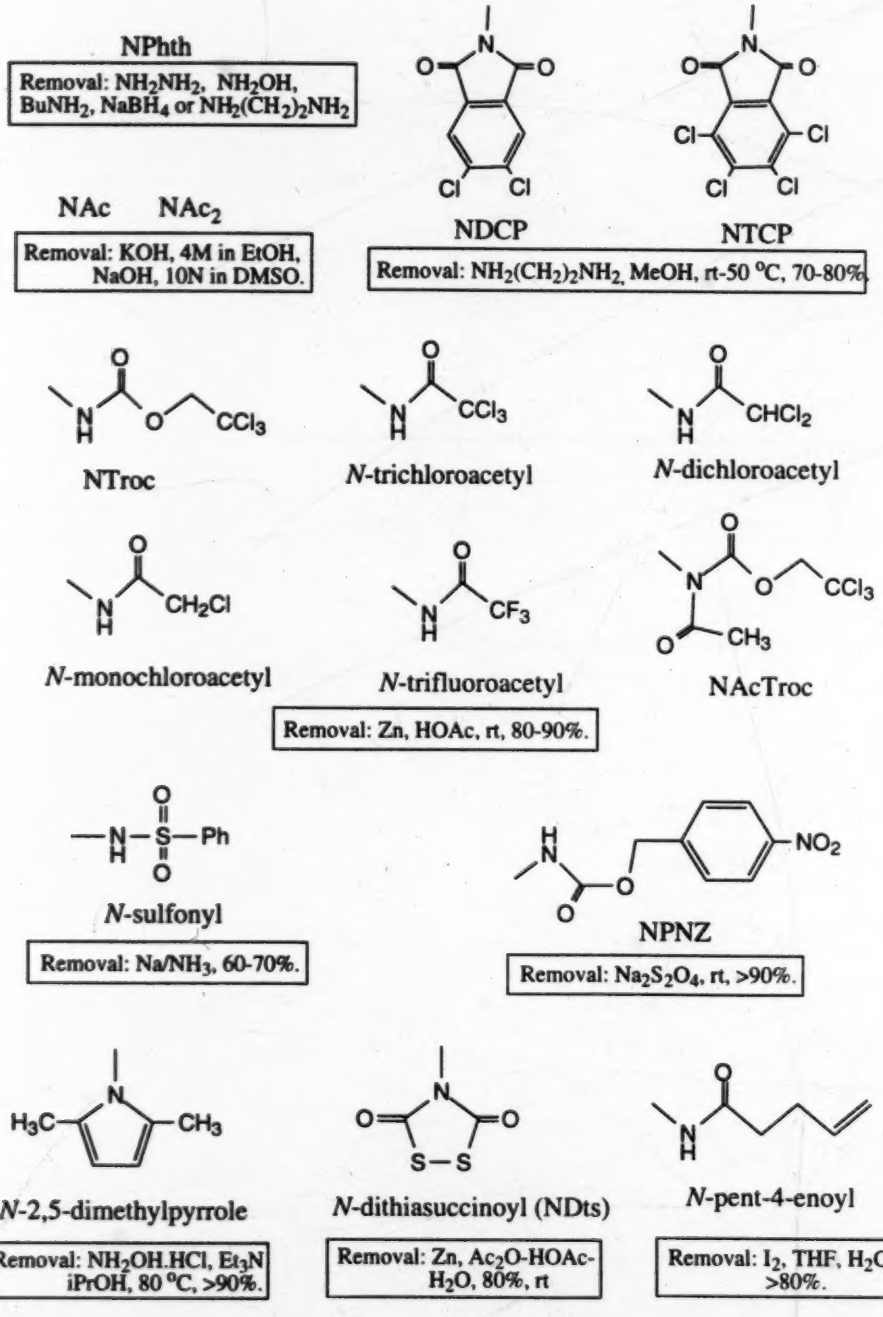


Fig. 4.4: Structures and conditions for removal of common amino protecting groups.

exception of the *N*-2,5-dimethylpyrroyl group. These protecting groups have proven very useful.

4.2.3. 1,2-*cis*-Glycosylations with 2-Amino-2-deoxy Sugars.

The synthesis of 1,2-*cis*-glycosides requires a non-participating group for amino protection. The azido group is the most widely used nonparticipating group. Reduction of the azido group gives the free amine [139, 140].

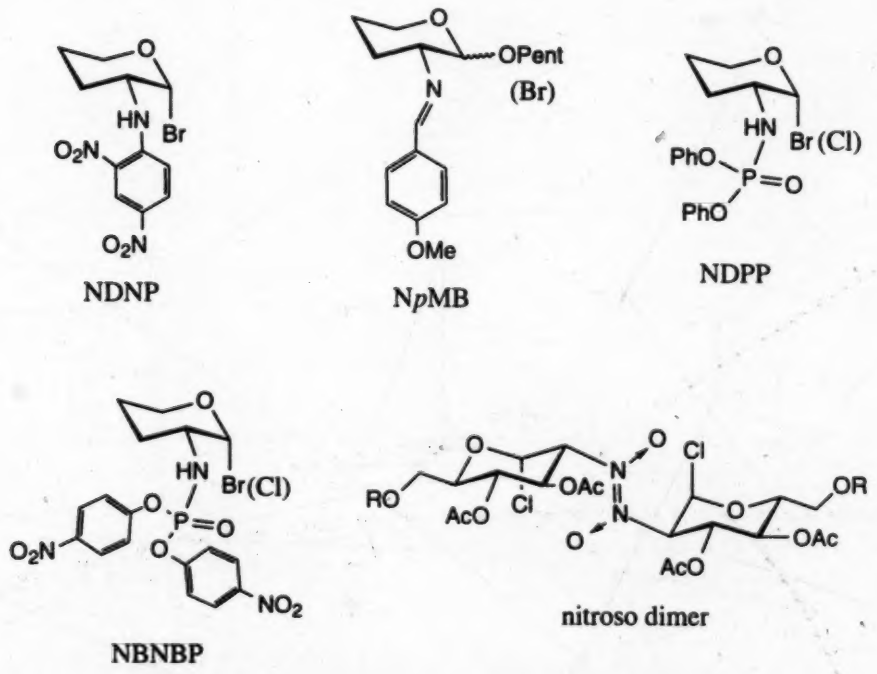
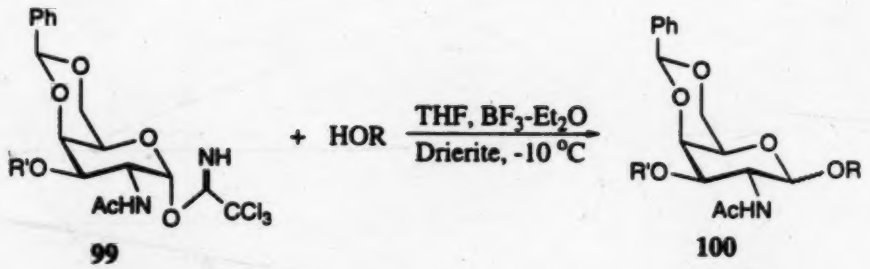


Fig. 4.6: Some nonparticipating amino protecting groups.

Several other nonparticipating amino protecting groups have been reported for the synthesis of 1,2-*cis*-glycosides. These groups are generally bulky and the stereoselectivity of the glycosylation reactions is accordingly usually poor. These non-participating amino

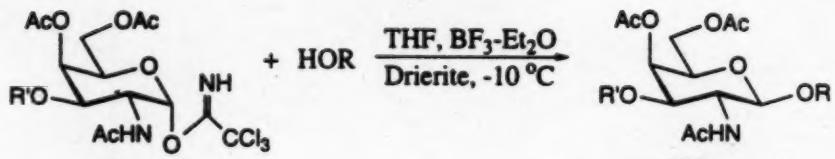


99 **100**

R' = Ac, Bz, C(NH)CCl$_3$, Sugars

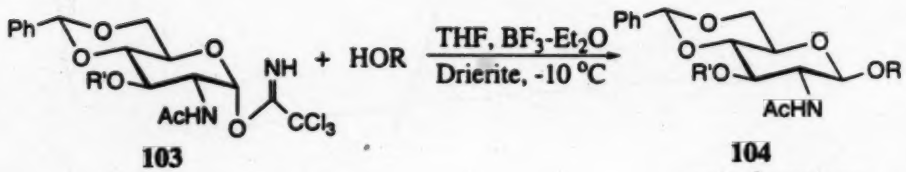
R = alcohols, serine, threonine

α/β > 9:1
yields >60%



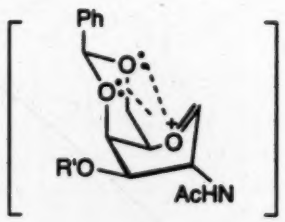
101 **102**

only β products



103 **104**

only β products



105

Scheme 4.2: Synthesis of α-*N*-acetylgalactosaminyl peptides.

protecting groups are *N*-2,4-dinitrophenyl (NDNP) [131, 141], *N*-*p*-methoxybenzylidene (N*p*MB) [142], *N*-diphenylphosphoryl (NDPP) [143], *N*-bis-[*p*-nitrobenzyl]-phosphoryl (NBNBP) [143], and a nitroso dimer derivative [144] (Fig. 4.6).

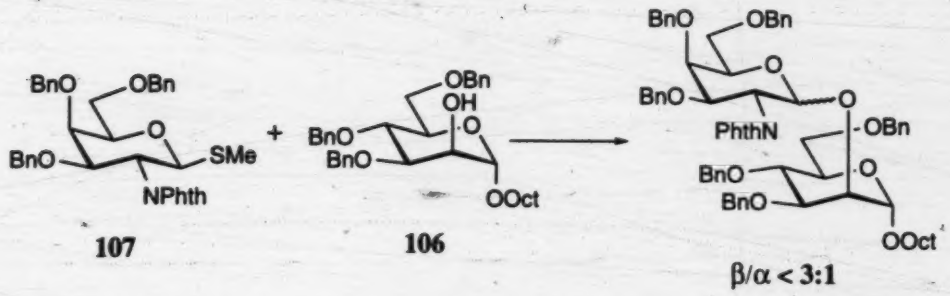
Although the stereoselectivity of glycosylation reactions is influenced by factors such as the solubility of the promotor in the reaction solvent and the amino protecting group, unexpected results are frequently obtained. For example, using different protected GalNAc or GlcNAc donors in coupling to amino acids (serine or threonine) resulted in different α/β selectivities (Scheme 4.2) [145]. The unexpected α-selectivity of **99** was attributed to the cyclic 1,3-dioxane ring which positions the 4-O and 6-O lone pairs to stabilize the oxycarbonium ion **105** that was hypothetical as intermediale in the reaction.

4.3. Objective.

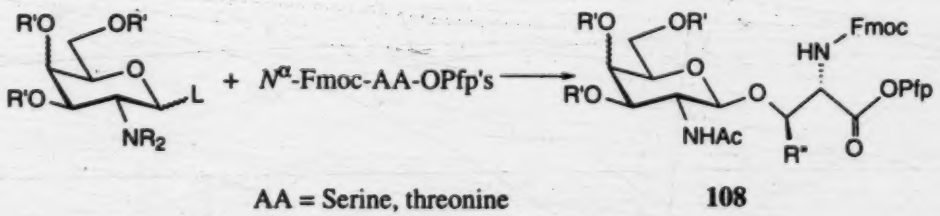
4.3.1. Creating a Novel Amino Protecting Group for the Synthesis of 1,2-*trans*-Glycosides.

As summarized above, more than a dozen amino protecting groups have been developed for the 1,2-*trans*-glycosylation of 2-amino sugars. However, most of the glycosylation reactions using these protecting groups proceed via the same type of intermediate (Fig. 4.5) so they offer no solutions in difficult cases. For example, in the course of a program on the preparation of 1,2-*trans*-linked oligosaccharide analogs, it was found that attempted synthesis of the β-GalNPhth-(1→2)-α-Man linkage resulted in an unusually high proportion of α-linked disaccharide despite the expected participation of the NPhth group. A similar situation had been previously encountered [146]. A β:α mixture (< 3:1, 75% yield) was formed on glycosylation of acceptor **106** with the tri-*O*-benzyl-NPhth donor **107** (Scheme 4.3). This was presumably due to a "mismatch" [147] in the

donor-acceptor pair. Also, in the synthesis of *O*-linked glycopeptides using solid phase methods, the preparation of building blocks of a glycosyl "active ester", glycosyl N^{α}-(9-fluorenylmethyloxycarbonyl)amino acid pentafluorophenyl ester (N^{α}-Fmoc-AA-OPfp's) **108** in Scheme 4.4, has special problems arising from the 2-amino substituent in the



Scheme 4.3: Synthesis of β-GalNPhth-(1→2)-α-Man with high proportion of α-linkage.



Scheme 4.4: Synthesis of 2-NAc-β-Glycosyl N^{α}-Fmoc-AA-OPfp's.

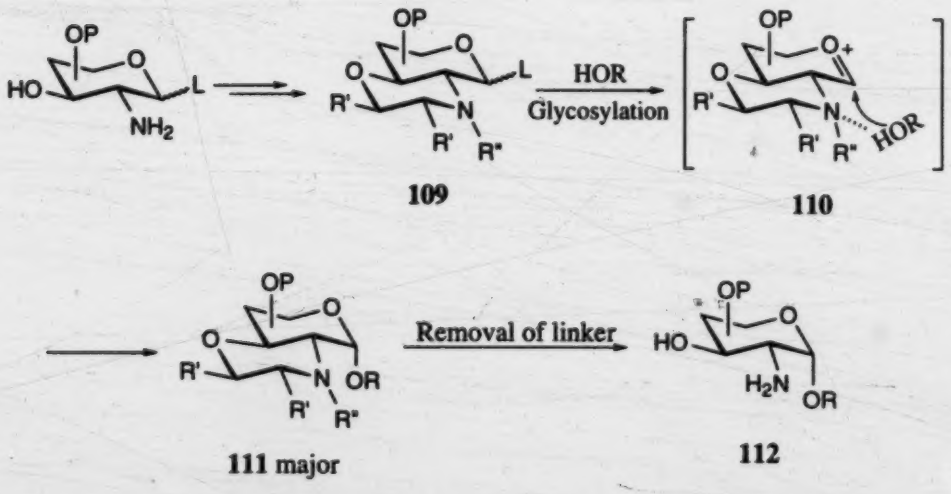
corresponding glycosyl donors [138]. Activation of donors with a 2-*N*-acyl group provides relatively unreactive oxazoline intermediates (Fig. 4.5), whereas the alternative phthaloyl (Phth) group requires prolonged base treatment at high temperatures for its

removal, and incomplete deprotection is often encountered. Strongly basic conditions also run the risk of epimerizing amino acids.

We therefore sought out an alternative donor that would not result in an intermediate similar to the oxazolinium ion (Fig. 4.5). Such a donor should be relatively reactive, would result in high 1,2-*trans* stereoselectivity and would be readily removed under very mild conditions.

4.3.2. Studies on 1,2-*cis*-Glycosylation of 2-Amino Sugars.

The most widely used method for the synthesis of 1,2-*cis*-glycosides of 2-amino sugars employs glycosyl donors with nonpaticipating group for amino protection. The azido functionality has been the favored nonparticipating group since it displays low steric hindrance. Lemieux and co-workers introduced the azido group for the synthesis of 1,2-*cis*-glycosides of 2-amino sugars in 1979 [139, 140]. Since then, many methods for the



Scheme 4.5: Glycosylation mechanism using new kind of donor with a 1,3-linker.

preparation of 2-azido glycopyranosides have been reported in the literature [108]. A major problems with these methods is that many steps are required for the synthesis of the azido-donors, which are obtained in low overall yields. As discussed earlier, other nonparticipating amino protecting groups are very bulky and glycosylation reactions using these donors result in very poor stereoselectivity. We therefore investigated a new strategy for amino group protection via tethering (see **109**, scheme 4.5) for the synthesis of 1,2-*cis*-glycosides of 2-amino sugars. The 2-*N*,3-*O* linker should prevent the amino group from participating effectively. This might lead to 1,2-*cis*-glycosylation due to the anomeric effect and the hydrogen bond formation between donor nitrogen and acceptor hydroxyl group (Scheme 4.5).

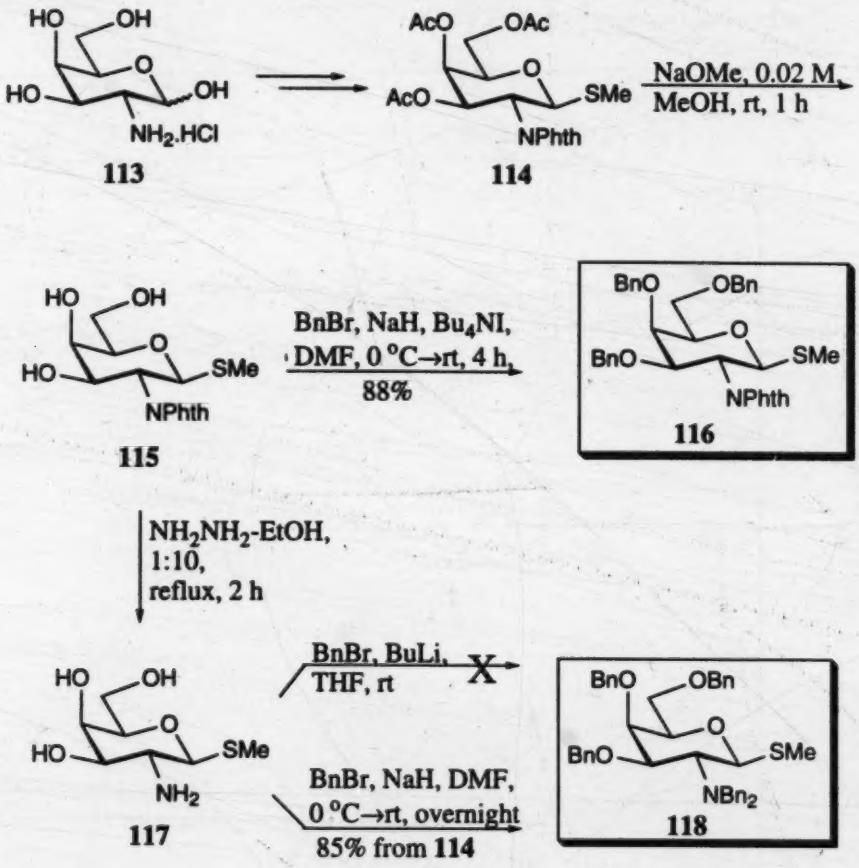
Chapter 5

The 2-*N*,*N*-Dibenzylamino Group as a Participating Group in the Synthesis of β-Glycosides

5.1. Introduction.

5.1.1. Synthesis of 2-*N*,*N*-Bibenzylamino and 2-Phthalimido Thiogalactopyranosyl Donors 118 and 116.

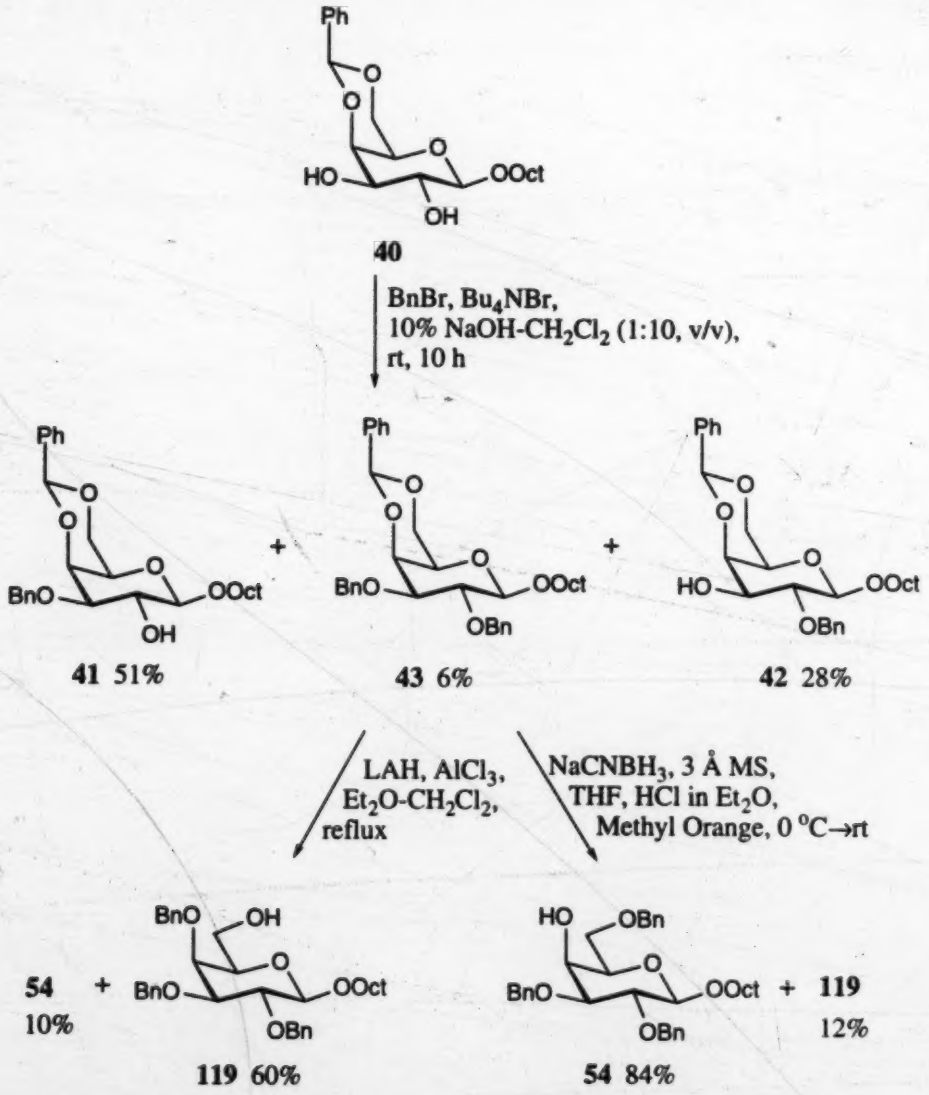
2-*N*,*N*-Dibenzylamino and 2-phthalimido thiogalactopyranoside donors **118** and **116** were prepared as shown in Scheme 5.1. Deacetylation of **114** (which was prepared as described in Part I of this thesis) using 0.02 M NaOMe in methanol at rt for 1 h afforded **115**. Removal of the phthalimido group in **115** with 10% hydrazine in refluxing ethanol for 2 h yielded the free amine **117**. Simultaneous *O*- and *N*-benzylation of **117** with BnBr and NaH in DMF at 0 °C to rt provided the 2-*N*,*N*-dibenzylamino thioglycoside donor **118** in 85% overall yield (from **114**). Simultaneous *O*- and *N*-benzylation of **117** with BnBr and BuLi in THF at rt failed to give the desired 2-*N*,*N*-dibenzylamino donor **118**. Treatment of **115** with BnBr and NaH in the presence of Bu_4NI in DMF at 0 °C to rt for 4 h, however, did produce the 3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido thioglycosyl donor **116** in high yield (88%).



Scheme 5.1: Preparation of glycosyl donors with 2-*N,N*-dibenzylamino and 2-NPhth groups.

5.1.2. Preparation of Partially Protected Octyl Galactopyranoside Acceptors.

The preparation of partially protected 2-, 3-, 4-, and 6-OH octyl galactopyranoside acceptors **41**, **42**, **119** and **54** is shown in Scheme 5.2. Monobenzylation of octyl 4,6-*O*-benzylidene-β-D-galactopyranoside (**40**) under phase-transfer catalysis conditions [69] using benzyl bromide in the presence of tetrabutylammonium bromide in a mixture of 10% aq NaOH and CH_2Cl_2 (1:10, v/v) yielded the 3-*O*-benzyl product **41** (51%), the 2-*O*-



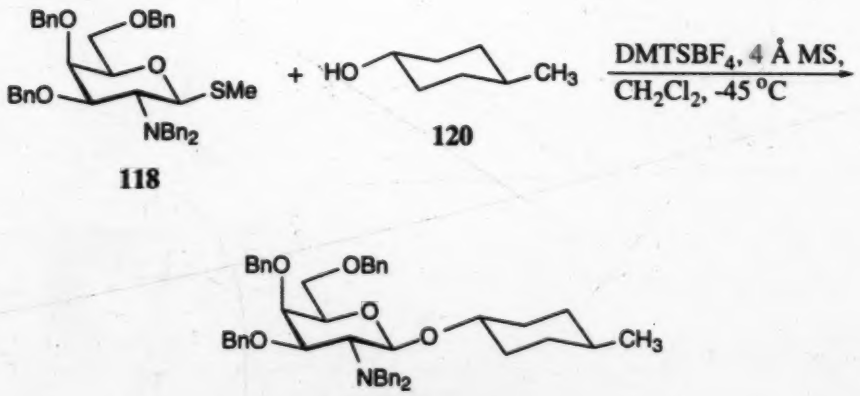
Scheme 5.2: Preparation of 2-, 3-, 4- and 6-OH octyl galactopyranosyl acceptors.

benzyl product **42** (28%) and the 2,3-di-*O*-benzyl product **43** (6%). Benzylidene ring opening of **43** with sodium cyanoborohydride-hydrogen chloride in the presence of 4 Å molecular sieves in THF at 0 °C to rt produced the 2,3,6-tri-*O*-benzyl galactopyranoside acceptor **54** in 84% yield and the 2,3,4-tri-*O*-benzyl acceptor **119** in 12% yield (see Part I

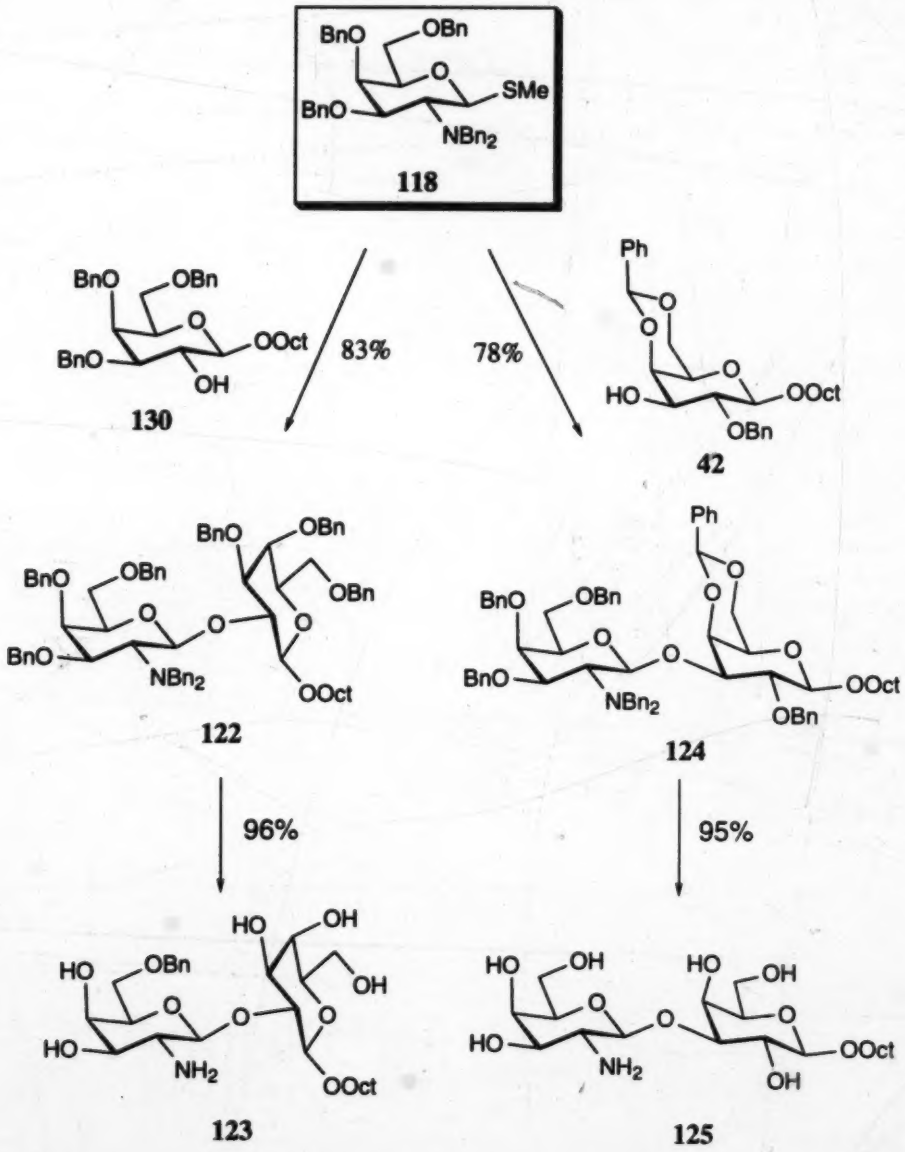
of this thesis for details). Alternatively, opening the benzylidene ring of **43** using lithium aluminum anhydride and aluminum chloride in refluxing Et_2O-CH_2Cl_2 gave **119** (60%) and **54** (10%) [148]. The position of the free hydroxyl groups in compounds **54** and **119** were confirmed via the ^1H NMR spectra of acetylated derivatives.

5.1.3. Evaluation of the Novel Glycosyl Donor 118.

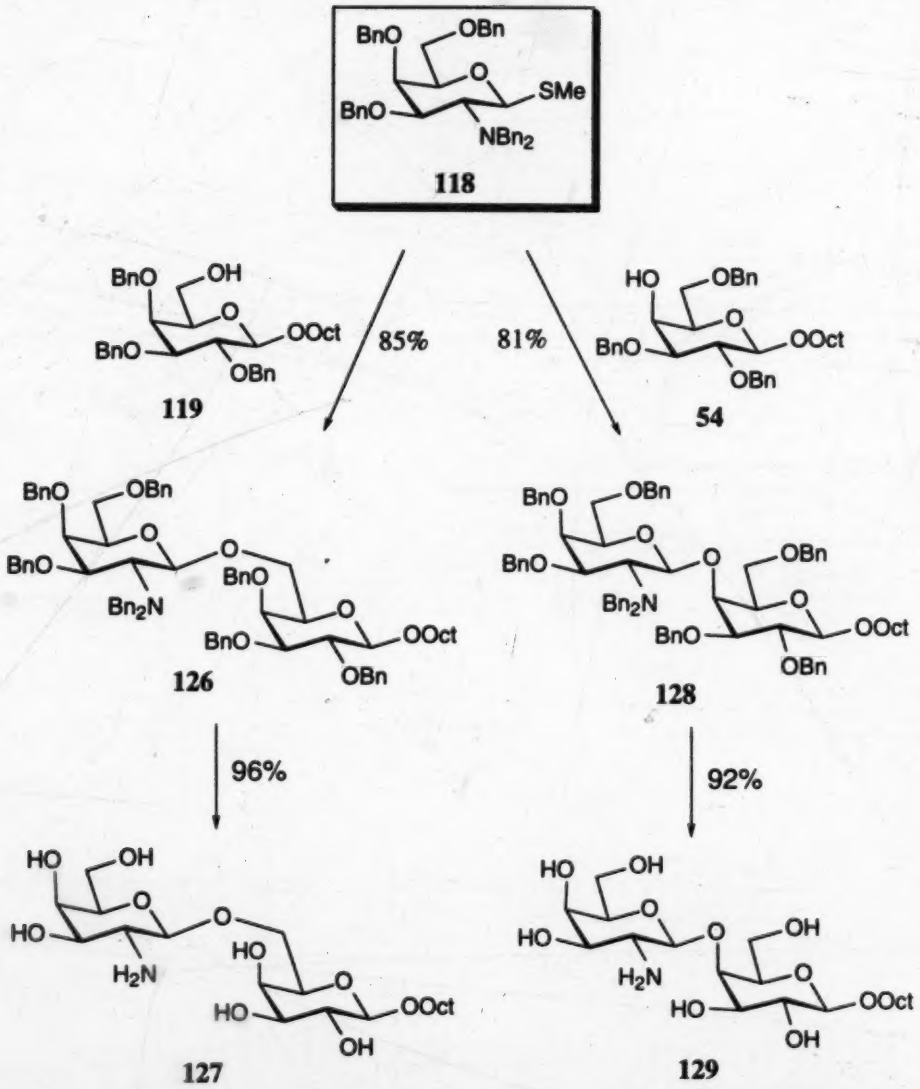
To evaluate the 2-*N,N*-dibenzylamino derivative **118** as a glycosyl donor we reacted it initially **118** with *trans*-4-methyl-cyclohexanol (**120**). This alcohol was chosen since its methyl group has a well resolved doublet signal that is readily integrated in the ^1H NMR spectrum. The neutral promotor dimethyl(methylthio)sulfonium tetrafluoroborate ($DMTSBF_4$) [149, 150] was used in the presence of 4 Å molecular sieves in CH_2Cl_2 at -45 °C. Only the β-product **121** was obtained in 82% yield (based on **118**) as shown in Scheme 5.3.



Scheme 5.3: Glycosylation of *trans*-4-methylcyclohexanol with donor **118**.



Scheme 5.4: Glycosylations of glycosyl acceptors **130** and **42** with donor **118**. *Conditions*: Glycosylation: **118** (2 equiv), $DMTSBF_4$ (4 equiv), 4 Å molecular seives, CH_2Cl_2, -30→0 °C, 2 h; Deprotection: $Pd(OH)_2/C$, H_2, HCl (0.2%), EtOH, 3 h.



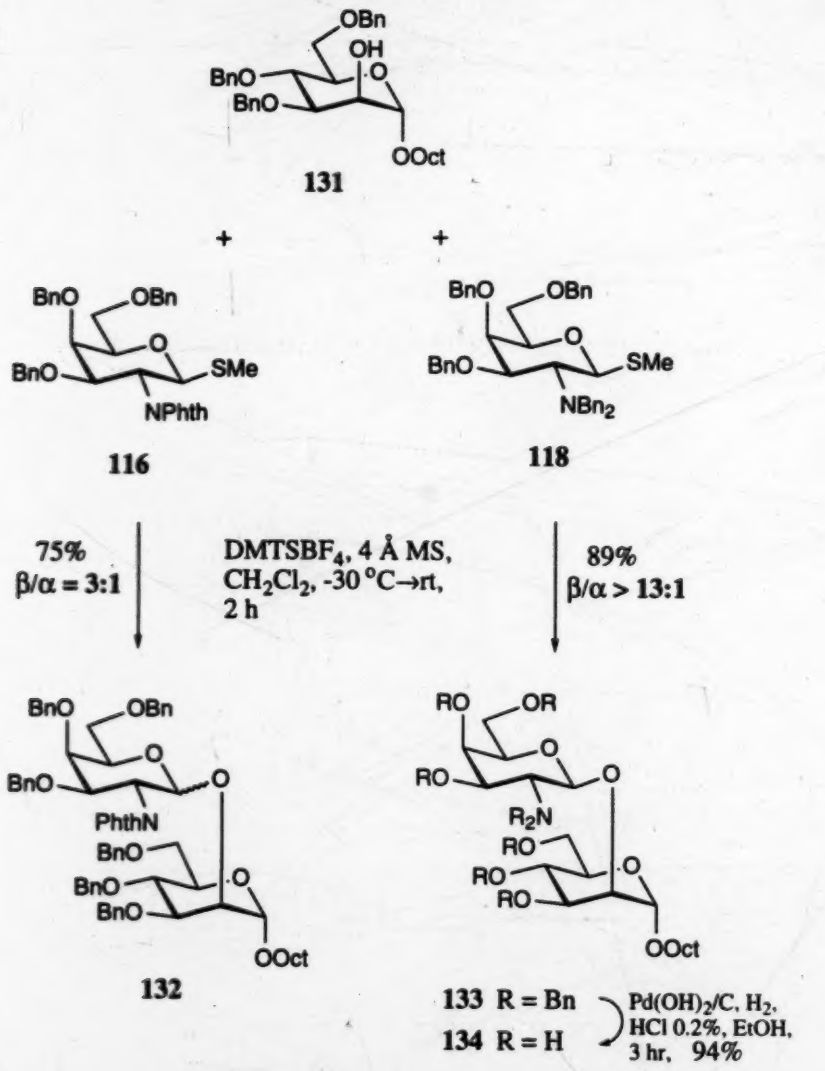
Scheme 5.4: Glycosylations of glycosyl acceptors **119** and **54** with donor **118** (contd). *Conditions*: Glycosylation: **118** (2 equiv), $DMTSBF_4$ (4 equiv), 4 Å molecular seives, CH_2Cl_2, -30→0 °C, 2 h; Deprotection: $Pd(OH)_2/C$, H_2, HCl (0.2%), EtOH, 3 h.

We then investigated the behavior of donor **118** in more challenging situations with the partially protected galactopyranosides **42**, **54**, **119** and **130** which present a spectrum of acceptor reactivities (Scheme 5.4). The glycosylation was carried out in a range of

solvents with CH_2Cl_2 being preferred (Table 5.1). Reaction in presence of $DMTSBF_4$ and 4 Å molecular sieves in CH_2Cl_2 at -30→0 °C gave the protected disaccharides **122**, **124**, **126** and **128** in good yields (over 78%) with high β-selectivities (β:α > 11:1) (Table 5.1). The amount of α anomer formed was estimated by integration of the H-1α signal which had a characteristic small coupling constant (4 Hz). The glycosylation rates and yields were similar for primary and secondary sugar alcohols. Hydrogenolysis of disaccharides **122**, **124**, **126** and **128** over Pearlman's catalyst in ethanol with 0.2% HCl smoothly removed both the *O*- and *N*-benzyl groups. Subsequent purification using C-18 Sep-Pak adsorption [93] gave the deprotected disaccharides **123**, **125** **127** and **129** in over 92% yields (Scheme 5.4).

entry	glycosyl donor	glycosyl acceptor	solvent	reaction time (h)	yield[a] (%)	β:α ratio[b]
1	**118**	**119**	CCl_4:CH_2Cl_2 (10:1)	10	67	2:1
2	**118**	**54**	CCl_4:CH_2Cl_2 (10:1)	10	54	10:1
3	**118**	**42**	CCl_4:CH_2Cl_2 (10:1)	10	59	3:1
4	**118**	**119**	MeCN:CH_2Cl_2 (3:1)	8	85	5:1
5	**118**	**54**	MeCN:CH_2Cl_2 (3:1)	8	62	11:1
6	**118**	**42**	MeCN:CH_2Cl_2 (3:1)	8	70	10:1
8	**118**	**119**	CH_2Cl_2	2	85	18:1
9	**118**	**54**	CH_2Cl_2	2	81	50:1
10	**118**	**42**	CH_2Cl_2	2	78	11:1
11	**118**	**130**	CH_2Cl_2	2	83	>50:1
12	**118**	**131**	CH_2Cl_2	2	86	13:1
13	**116**	**131**	CH_2Cl_2	2	75	3:1

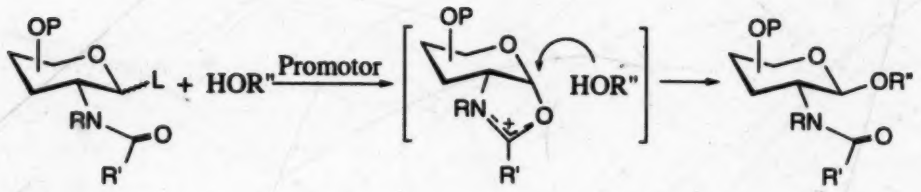
Table 5.1: Glycosylation results using **116** and **118** as donors with octyl galactopyranosyl acceptors in different solvents. a) Given for products purified by column chromatography and based on glycosyl acceptor; b) Determined by ^1H NMR analysis.



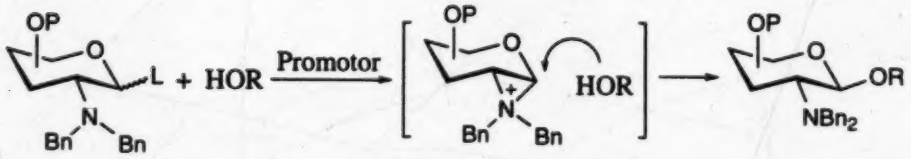
Scheme 5.5: Glycosylations of octyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside **131** with donors **116** and **118**.

Donor **118** was next evaluated as a glycosyl donor for the synthesis of the "mismatched" 2-amino-2-deoxy-β-D-galactopyranosyl-(1-2)-α-D-manopyranoside sequence. Condensation of **118** with octyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**131**) in the presence of $DMTSBF_4$ and 4 Å molecular sieves in dichloromethane at -30 to

0 °C (Scheme 5.5) gave the disaccharide with excellent β-selectivity (β:α ≥ 13:1) and in high yield (86%). One-step deprotection of **133** by hydrogenolysis gave the free amine **134** in 94% yield. In contrast, condensation of the 2-*N*-Phthalimido donor **116** with **131** gave a disaccharides in 75% yield and with poor β-selectivity (β:α = 3:1).



135



136

Scheme 5.6: Postulated glycosylation mechanisms for glycosyl donors with 2-*N*,*N*-dibenzylamino and 2-*N*-acylimido groups (P indicates a protecting group).

5.1.4. Conclusion.

In summary, the 2-*N*,*N*-dibenzylamino thioglycoside **118** is a new and mechanistically different glycosyl donor. The glycosidic linkage is formed with high β/α-stereoselectivity and in excellent yield for a range of challenging acceptors. Though we have no direct evidence, the intermidiacy of **136** (Scheme 5.6) may account for the net retention of configuration at C-1. The *N*-benzyl groups can be very efficiently removed by hydrogenolysis, obviating the need for harsh basic or other alternate chemical reactions for

N-deprotection. We propose that, because of the unique SP^3 hybridization of the nitrogen in donor **118**, such donors represent very useful alternatives when use of *N*-acylated donors result in low yields or poor stereoselectivities. The behavior of *N,N*-dibenzyl protected acceptors in glycosylation reactions is under investigation.

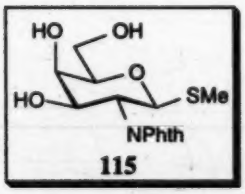
5.2. Experimental Section.

5.2.1. General Methods.

Same as described in Chapter 2, Part I. The preparations of **114**, **41**, **43**, **42**, **54**, and **131** were performed in the same ways as described in Part I.

5.2.2. Experimental.

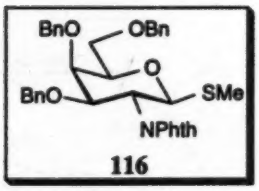
Methyl 2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside (**115**).



115

To a suspension of **114** (0.5 g, 1.07 mmol) in dry MeOH (30 mL), NaOMe (27 mg, about 0.02 M) was added at rt. The mixture was stirred for 1 h and TLC indicated complete reaction (R_f = 0.60, CH_2Cl_2-MeOH, 10:1). The solution was neutralized with Dowex 50W-X8 $[H]^+$ resin, filtered, and concentrated to give crude product **115** (quantitative yield). NMR data (CD_3OD): ^1H: δ 7.75-7.90 (m, 4 H, aromatic), 5.14 (d, 1 H, J = 10.0 Hz, H-1), 4.50 (t, 1 H, J = 10.0 Hz, H-2), 4.46 (dd, 1 H, J = 10.0, 3.0 Hz, H-3), 4.0 (dd, 1 H, J = 3.0, 1.0 Hz, H-4), 3.81 (dd, 1 H, J = 12.0, 7.0 Hz, H-6a), 3.75 (dd, 1 H, J = 12.0, 5.0 Hz, H-6b), 3.69 (ddd, 1 H, J = 7.0, 5.0, 1.0 Hz, H-5) and 2.14 (s, 3 H, Sme).

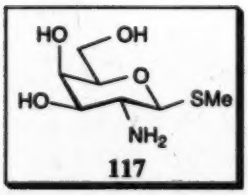
Methyl 3,4,6-tri-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside (**116**).



116

Crude compound **115** (190 mg, 0.56 mmol), NaH (85 mg, 80% in oil, 3.36 mmol) and Bu_4NI (1.24 g, 3.36 mmol) in DMF (20 mL) were stirred for 30 min at rt and then BnBr (0.4 mL, 3.36 mmol) was added. After 4 h, some MeOH was added to the reaction mixture to decompose the excess of NaH and then EtOAc (100 mL) was added. The solution was washed with brine (3 x 100 mL), dried with $MgSO_4$, filtered, and then concentrated. The residue was purified by column chromatography (hexane-EtOAc, 3.5:1) to give donor **116** (300 mg, 88%, R_f = 0.44, hexane-EtOAc, 2:1). ^1H NMR ($CDCl_3$): δ = 5.14 (d, 1 H, J = 10.5 Hz, H-1), 4.98 (d, 1 H, J = 11.5 Hz, O*CH*Ph), 4.85 (t, 1 H, J = 10.5 Hz, H-2), 4.60 (d, 2 H, J = 12.0 Hz, 2 x O*CH*Ph), 4.51 and 4.46 (2 d, 2 H, J = 11.5 Hz, O*CH*$_2$Ph), 4.39 (dd, 1 H, J = 11.0, 2.8 Hz, H-3), 4.32 (d, 1 H, J = 12.0 Hz, O*CH*Ph), 4.10 (d, 1 H, J = Hz, H-4), 3.83 (t, 1 H, J = 6.5 Hz, H-5), 3.66 (m, 2 H, 2 x H-6) and 2.15 (s, 3 H, SMe); ^{13}C NMR ($CDCl_3$): δ = 168.43, 167.86, 138.78, 137.94, 137.72, 134.04, 133.93, 131.74, 129.06, 128.47, 128.36, 128.24, 127.96, 127.92, 127.84, 127.60, 127.49, 123.53, 123.40, 123.17, 80.76, 77.53, 77.39, 74.53, 73.55, 72.37, 71.51, 68.50, 51.04 and 11.05. HR-MS(ES): 610.2188 $[M+H]^+$

Methyl 2-amino-2-deoxy-1-thio-β-D-galactopyranoside (**117**).

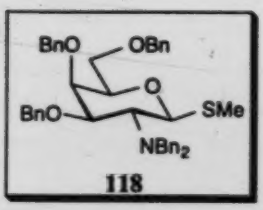


117

A solution of crude compound **115** (300 mg) in hydrazine monohydrate-ethanol (1:10, 50 mL) was refluxed for 1 h and then concentrated. The residue was coevaparoted with ethanol (2 x 30 mL) and toluene (2 x 30 mL) to yield crude product **117** (R_f =

0.24, 3:1, CH_2Cl_2-MeOH containing 1% of Et_3N). NMR data (CD_3OD): ^1H: δ = 4.37 (d, 1 H, J = 10.0 Hz, H-1), 3.89 (dd, 1 H, J = 3.5, 1.0 Hz, H-4), 3.76 (dd, 1 H, J = 11.0, 7.4 Hz, H-6a), 3.68 (dd, 1 H, J = 11.0, 4.8 Hz, H-6b), 3.64 (ddd, 1 H, J = 7.4, 4.8, 1.0 Hz, H-5), 3.60 (dd, 1 H, J = 10.0, 3.5 Hz, H-3), 3.13 (t, 1 H, J = 10.0 Hz, H-3), 2.23 (s, 3 H, SMe).

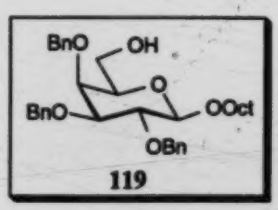
Methyl 3,4,6-tri-O-benzyl-2-deoxy-2-N,N-dibenzylamino-1-thio-β-D-galactopyranoside (**118**).



118

To a solution of crude compound **117** (300 mg, 1.4 mmol) in DMF (25 mL) at 0 °C was added NaH (430 mg, 80% in oil, 14.4 mmol). The mixture was stirred for 30 min at rt and then BnBr (1.3 mL, 10.8 mmol) was added. After 10 h, some MeOH was added to the reaction solution to decompose the excess of NaH and then EtOAc (100 mL) was added. The mixture was washed with Brine (3 x 100 mL), dried with $MgSO_4$, filtered, and then concentrated. The residue was subjected to column chromatography (hexane-EtOAc, 10:1) to give thioglycoside **118** (850 mg, 90%, R_f = 0.47, hexane-EtOAc, 5:1). ^1H NMR ($CDCl_3$): δ = 4.85 (d, 2 H, J = 11.5 Hz, 2 x O*CH*Ph), 4.61 (d, 1 H, J = 11.5 Hz, O*CH*Ph), 4.55 (d, 1 H, J = 11.5 Hz, O*CH*Ph), 4.48 and 4.43 (2 d, 2 H, J = 11.5 Hz, O*CH*$_2$Ph), 4.32 (d, 1 H, J = 10.0 Hz, H-1), 4.15 (d, 1 H, J = 2.5 Hz, H-4), 3.86 (dd, 1 H, J = 10.5, 2.5 Hz, H-3), 3.79 (b, 4 H, 2 x N*CH*$_2$Ph), 3.65-3.58 (m, 2 H, 2 x H-6), 3.47 (m, 1 H, H-5), 3.45 (t, 1 H, J = 10.0 Hz, H-2), and 1.90 (s, 3 H, SMe); ^{13}C NMR ($CDCl_3$): δ = 140.05, 138.86, 138.00, 129.66, 128.58, 128.50, 128.12, 128.01, 127.93, 127.87, 127.79, 127.37, 126.86, 85.39, 82.22, 76.64, 74.27, 73.68, 72.21, 70.58, 68.87, 58.15 and 12.15; HR-MS(ES): 660.3146 $[M+H]^+$.

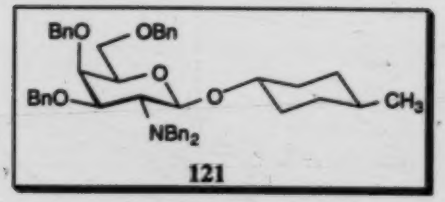
Octyl 2,3,4-tri-O-benzyl-β-D-galactopyranoside (**119**).



119

To a solution of **43** (1.4 g) in Et_2O-CH_2Cl_2 (1:1, 30 mL), $LiAlH_4$ (400 mg) was added in three portions with stirring, and the mixture was slowly heated to the boiling point. To the hot solution, $AlCl_3$ (1.5 g) in Et_2O (15 mL) was added during 30 min, and boiling was continued for 2 h. After TLC indicated the absence of starting material, the mixture was cooled, the excess of $LiAlH_4$ was decomposed with some EtOAc, and $Al(OH)_3$ was precipitated by the addition of water (6 mL). The solution was diluted with Et_2O (50 mL), separated, and the residue was washed with little Et_2O. The organic phase was washed with Brine (3 x 20 mL), dried with $MgSO_4$, filtered, and then concentrated. The residue was subjected to column chromatography (hexane-EtOAc, 3:1) to give product **119** (840 mg, 60%, R_f = 0.40, hexane-EtOAc, 2:1) and **54** (140 mg, 10%). ^{1}H NMR ($CDCl_3$): δ = 7.35-7.20 (m, 15 H, aromatics), 4.89 and 4.60 (2 d, 2 H, J = 12.0 Hz, O*CH*$_2$Ph), 4.88 and 4.71 (2 d, 2 H, J = 12.0 Hz, O*CH*$_2$Ph), 4.76 and 4.67 (2 d, 2 H, J = 12.0 Hz, O*CH*$_2$Ph), 4.29 (d, 1 H, J = 7.8 Hz, H-1), 3.90-3.83 (m, 1 H, O*CH*$(CH_2)_6$), 3.77 (dd, 1 H, J = 10.0, 7.8 Hz, H-2), 3.70 (bq, 2 H, 2 x H-6), 3.50-3.40 (m, 3 H, H-3, H-4, O*CH*$(CH_2)_6$), 3.30 (td, J = 6.0, 1.0 Hz, H-5), 1.65-1.55 (m, 2 H, $OCH_2CH_2(CH_2)_5$), 1.30-1.15 (m, 10 H, $O(CH_2)_2(CH_2)_5CH_3$), and 0.80 (t, 3 H, $O(CH_2)_7CH_3$). Acetylated **II-24**: Selected ^{1}H NMR ($CDCl_3$): δ = 3.34 (d, 1 H, J = 7.8 Hz, H-1), 4.22 (dd, 1 H, J = 11.0, 6.5 Hz, H-6a), 4.06 (dd, 1 H, J = 11.0, 6.5 Hz, H-6b), and 3.77 (bd, 1 H, J = 3.5 Hz, H-4).

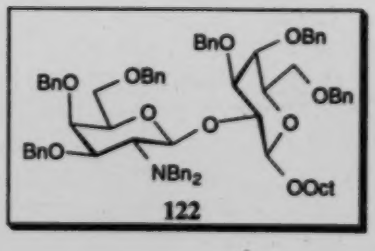
trans-(4-Methyl)cyclohexyl 3,4,6-tri-O-benzyl-2-deoxy-2-N,N-dibenzylamino-β-D-galactopyranoside (**121**).



121

A mixture of glycosyl donor **118** (23 mg, 0.035 mmol), *trans*-4-cyclohexanol (**120**) (8.7 µL) and 4 Å molecular sieves (250 mg) in CH_2Cl_2 (1 mL) was stirred for 1 h at rt, cooled to -30 °C and then $DMTSBF_4$ (13.7 mg, 0.07 mmol) was added under Ar. The temperature was increased to 0 °C slowly over 2 h. TLC showed complete disappearance of donor **118**. The mixture was filtered through Celite and the Celite was washed with CH_2Cl_2. The organic solution was concentrated. The residue was purified with chromatographic column (hexane-EtOAc, 10:1) to give the β-linked product **121** (21 mg, 82%, R_f = 0.50, hexane-EtOAc, 4:1). ^{1}H NMR ($CDCl_3$): δ = 7.50-7.00 (m, 25 H, aromatics), 4.77 and 4.54 (2 d, 2 H, J = 11.5 Hz, O*CH*$_2$Ph), 4.74 and 4.42 (2 d, 2 H, J = 11.5 Hz, O*CH*$_2$Ph), 4.48 and 4.46 (2 d, 2 H, J = 12.0 Hz, O*CH*$_2$Ph), 4.62 (d, 1 H, J = 8.2 Hz, H-1), 3.97 (bd, J = 2.0 Hz, H-4), 3.92 (s, 4 H, 2 x N*CH*$_2$Ph), 3.62-3.52 (m, 4 H, H-3, 2 x H-6, and O*CH*$(CH_2)_4CHCH_3$), 3.44 (t, 1 H, J = 7.0 Hz, H-5), 3.37 (dd, 1 H, J = 10.5, 8.2 Hz, H-2), 2.20-2.04, 1.82-1.64, 1.52-1.36, and 1.02-0.90 (m, 9 H, OCH*(CH*$_2)_4$*CH*CH_3) and 0.88 (d, 3 H, CH*CH*$_3$).

Octyl 2-O-(3,4,6-tri-O-benzyl-2-deoxy-2-N,N-dibenzylamino-β-D-galactopyranosyl)-3,4,6-tri-O-benzyl-β-D-galactopyranoside (**122**).

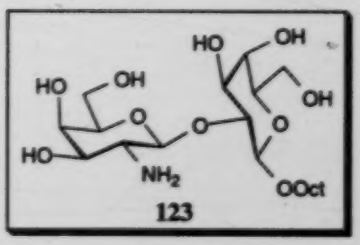


122

A mixture of glycosyl donor **118** (7 mg, 10.6 µmol), **130** (3 mg, 5.3 µmol) and 4 Å molecular sieves (100 mg) in CH_2Cl_2 (0.5 mL) was stirred for 2 h at rt, cooled to -30 °C and then $DMTSBF_4$ (4.2 mg, 21.2 µmol) was added under Ar. The temperature was increased to 5 °C slowly over 2 h. TLC showed complete disappearance of donor **118**. The mixture was filtered through Celite and then the Celite was washed with CH_2Cl_2. The

CH_2Cl_2 solution was concentrated and the residue was purified with chromatographic column (3% acetone in toluene) to give product **122** (4.5 mg, 83%, recovered 0.8 mg of **130**, β/α > 50:1, R_f = 0.45, toluene-acetone, 19:1). Selected ^{1}H NMR ($CDCl_3$): δ = 5.01 (d, 1 H, J = 8.0 Hz, H-1') and 4.51 (d, 1 H, J = 7.8 Hz, H-1), 4.25 (dd, 1 H, J = 7.8, 10.0 Hz, H-2), 4.0 (bd, 1 H, J = 2.0 Hz, H-4), 3.92 and 3.83 (2 d, 4 H, J = 13.5 Hz, 2 x N*CH*$_2$Ph) and 3.43 (dd, 1 H, J = 8.0, 10.0 Hz, H-2'); ^{13}C NMR ($CDCl_3$): δ = 140.67, 138.88, 138.58, 138.51, 138.08, 128.99, 128.46, 129.29, 129.24, 128.18, 128.10, 128.00, 127.92, 127.86, 127.47, 127.37, 127.26, 127.15, 127.03, 126.36, 102.60 and 97.95 (C-1', C-1), 74.37, 73.68, 73.55, 73.01, 72.86, 72.48, 72.04, 71.48, 69.91, 68.74, 29.88, 29.75, 29.55, 29.32 and 22.73.

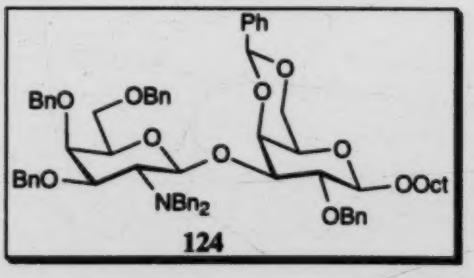
Octyl 2-O-(2-deoxy-2-amino-β-D-galactopyranosyl)-β-D-galactopyranoside (**123**).



123

A suspension of disaccharide **122** (3.5 mg), $Pd(OH)_2$/C (10%, 4 mg) in EtOH (2 mL) with HCl (0.2%) was stirred under H_2 at rt for 3 h. TLC showed complete disappearance of starting material. The mixture was then filtered through a Millex-GV filter unit and then the filter unit was washed with MeOH. The MeOH solution was concentrated and the residue was purified using a C-18 Sep-Pak cartridge to give product **123** (1.3 mg, 96%, R_f = 0.42, CH_2Cl_2-MeOH-NH_4OH, 4:3:1). Selected physical data for **123**: ^{1}H NMR (D_2O): δ = 4.51 (d, 1 H, J = 7.9 Hz, H-1') and 3.0 (dd, 1 H, H-2'); HR-MS(ES): 454.2695 $[M+H]^+$.

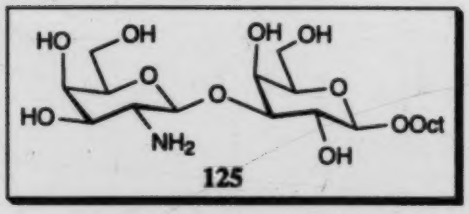
Octyl 3-O-(3,4,6-tri-O-benzyl-2-deoxy-2-N,N-dibenzylamino-β-D-galactopyranosyl)-2-O-benzyl-4,6-O-benzylidene-β-D-galactopyranoside (**124**).



124

A mixture of glycosyl donor **118** (7 mg, 10.6 μmol), **42** (2.5 mg, 5.3 μmol) and 4 Å molecular sieves (100 mg) in CH_2Cl_2 (0.5 mL) was stirred for 1 h at rt, cooled to -30 °C and then $DMTSBF_4$ (4.2 mg, 21.2 μmol) was added under Ar. The temperature was increased to 6 °C slowly over 3 h. TLC showed complete disappearance of donor **118**. The mixture was filtered through Celite and then the Celite was washed with CH_2Cl_2. The CH_2Cl_2 solution was concentrated and the residue was purified with chromatographic column (4% acetone in toluene) to give product **124** (3.0 mg, 78%, recovered 0.8 mg of **42**, β/α > 11:1, R_f = 0.33, toluene-acetone, 19:1). ^{1}H NMR ($CDCl_3$): δ = 7.70-7.00 (m, 35 H, aromatics), 5.52 (s, 1 H, O_2CHPh), 5.25, 4.73, 4.70, and 4.67 (4 d, 4 H, J = 12.0 Hz, 2 x OCH_2Ph), 5.06 (d, 1 H, J = 8.0 Hz, H-1'), 4.50 and 4.28 (m, 4 H, 2 x OCH_2Ph), 4.58 (d, 1 H, J = 8.0 Hz, H-1), 4.25 (bs, 1 H, H-4), 4.05-3.95 (m, 4 H, 2 x H-6, H-5, and $OCH(CH_2)_6$), 3.90 (sb, 1 H, H-4'), 3.88, and 3.78 (2d, 4 H, j = 14.0 Hz, 2 x NCH_2Ph), 3.60-3.40 (m, 6 H, 2 x H-6', H-5', H-3', H-2, H-2'), 3.35-3.30 (m, 2 H, H-3, and $OCH(CH_2)_6$), 1.65-1.55 (m, 2 H, $OCH_2CH_2(CH_2)_5$), 1.40-1.15 (m, 10 H, $O(CH_2)_2(CH_2)_5CH_3$), and 0.85 (t, 3 H, $O(CH_2)_7CH_3$); ^{13}C NMR ($CDCl_3$): δ = 140.34, 139.35, 138.15, 128.84, 128.52, 128.27, 128.07, 127.84, 127.35, 127.19, 126.64, 126.52, 126.25, 104.41, 103.00 and 101.00 (C-1', C-1), 79.61, 79.04, 74.86, 74.45, 73.71, 73.52, 74.40, 72.91, 71.73, 70.06, 69.16, 69.03, 66.74, 58.86, 31.84, 29.71, 29.45, 29.23, 26.20, 22.69 and 14.13.

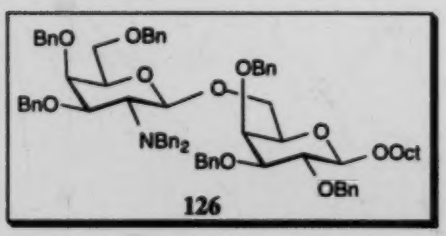
Octyl 3-O-(2-deoxy-2-amino-β-D-galactopyranosyl)-β-D-galactopyranoside (**125**).



125

A suspension of disaccharide **124** (5 mg), $Pd(OH)_2/C$ (10%, 5 mg) in EtOH (2 mL) with HCl (0.2%) was stirred under H_2 at rt for 3 h. TLC showed complete disappearance of starting material. The mixture was then filtered through a Millex-GV filter unit and then the filter unit was washed with methanol. The methanol solution was concentrated and the residue was purified using a C-18 Sep-Pak cartridge to give product **125** (2.0 mg, 95%, R_f = 0.52, CH_2Cl_2-MeOH-NH_4OH, 4:3:1). Selected physical data for **125:** ^1H NMR (D_2O): δ = 4.53 (d, 1 H, J = 8.1 Hz, H-1'), 4.43 (d, 1 H, J = 8.1 Hz, H-1) and 2.93 (dd, 1 H, H-2'); ^{13}C NMR (D_2O): δ = 103.10 ($J_{C1\text{-}H1}$ = 160.2 Hz, C-1), 105.90 ($J_{C1'\text{-}H1'}$ = 160.3 Hz, C-1'); HR-MS(ES): 454.2652 $[M+H]^+$.

Octyl 6-O-(3,4,6-tri-O-benzyl-2-deoxy-2-N,N-dibenzylamino-β-D-galactopyranosyl)-2,3,4-tri-O-benzyl-β-D-galactopyranoside (**126**).

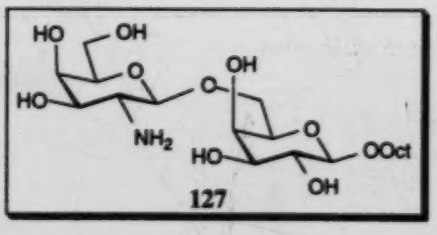


126

A mixture of glycosyl donor **118** (15 mg, 22.8 μmol), **119** (8.5 mg, 15.2 μmol) and 4 Å molecular sieves (200 mg) in CH_2Cl_2 (0.8 mL) was stirred for 1 h at rt, cooled to -50 °C and then $DMTSBF_4$ (9.0 mg, 45.6 μmol) was added under Ar. The temperature was increased to -30 °C slowly over 2 h. TLC showed complete disappearance of donor **118**. The mixture was filtered through Celite and then the Celite was washed with CH_2Cl_2. The CH_2Cl_2 solution was concentrated and the residue was purified with chromatographic column (4% acetone in toluene) to give product **126** (12.5 mg, 85%, recovered 1.5 mg of **119**, β/α > 18:1, R_f = 0.44, toluene-acetone, 19:1). ^1H NMR ($CDCl_3$): δ = 7.40-7.10 (m, 40 H, aromatics), 4.96, 4.89, 4.79, 4.70,

4.63, and 4.58 (6 d, 6 H, 3 x OCH_2Ph), 4.76 (d, 1 H, J = 8.0 Hz, H-1'), 4.50-4.40 (m, 6 H, 3 x OCH_2Ph), 4.42 (d, 1 H, J = 7.7 Hz, H-1), 4.05-3.95 (m, 3 H, H-4, 2 x H-6), 3.88 (s, 4 H, J = 14.0 Hz, 2 x NCH_2Ph), 3.84 (dd, 1 H, J = 7.7, 9.8 Hz, H-2), 3.80-3.40 (m, 9 H, 2 x H-6', H-5, O$CH_2(CH_2)_6$, H-4', H-5', H-3', and H-3), 3.35 (dd, 1 H, J = 8.0, 10.0 Hz, H-2'), 1.65-1.55 (m, 2 H, $OCH_2CH_2(CH_2)_5$), 1.40-1.15 (m, 10 H, $O(CH_2)_2(CH_2)_5CH_3$), and 0.85 (t, 3 H, $O(CH_2)_7CH_3$); ^{13}C NMR ($CDCl_3$): δ = 140.40, 138.94, 138.78, 138.58, 138.46, 137.98, 128.92, 128.52, 128.47, 128.43, 128.36, 128.29, 128.17, 128.13, 128.04, 127.97, 127.90, 128.80, 127.66, 127.50, 127.36, 127.29, 126.62, 104.10 and 102.49 (C-1', C-1), 82.20, 79.68, 79.59, 75.17, 74.41, 74.28, 74.13, 73.60, 73.28, 72.90, 72.28, 71.53, 70.28, 68.91, 68.44, 59.26, 31.82, 29.78, 29.47, 29.25, 26.17, 22.68 and 14.11.

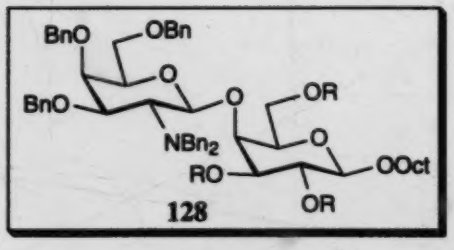
Octyl 6-O-(2-deoxy-2-amino-β-D-galactopyranosyl)-β-D-galactopyranoside (**127**).



127

A suspension of disaccharide **126** (5 mg), $Pd(OH)_2$/C (10%, 5 mg) in EtOH (2 mL) with HCl (0.2%) was stirred under H_2 at rt for 3 h. TLC showed complete disappearance of starting material. The mixture was then filtered through a Millex-GV filter unit and then the filter unit was washed with MeOH. The MeOH solution was concentrated and the residue was purified using a C-18 Sep-Pak cartridge to give product **127** (1.9 mg, 96%, R_f = 0.46, CH_2Cl_2-MeOH-NH_4OH, 4:3:1). Selected physical data for **127**: ^{1}H NMR (D_2O): δ = 4.72 (d, 1 H, J = 8.6 Hz, H-1'), 4.42 (d, 1 H, J = 8.1 Hz, H-1) and 3.22 (dd, 1 H, H-2'); HR-MS(ES): 454.2652 $[M+H]^+$.

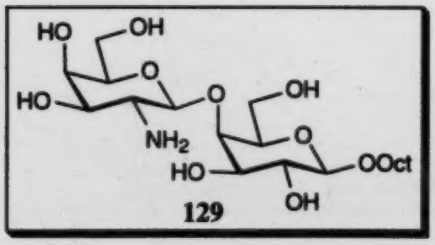
Octyl 4-O-(3,4,6-tri-O-benzyl-2-deoxy-2-N,N-dibenzylamino-β-D-galactopyranosyl)-2,3,4-tri-O-benzyl-β-D-galactopyranoside (**128**).



128

A mixture of glycosyl donor **118** (7.0 mg, 10.6 μmol), **54** (3.0 mg, 5.3 μmol) and 4 Å molecular sieves (100 mg) in CH_2Cl_2 (0.5 mL) was stirred for 1 h at rt, cooled to -30 °C and then $DMTSBF_4$ (4.2 mg, 21.2 μmol) was added under Ar. The temperature was increased to 6 °C slowly over 3 h. TLC showed complete disappearance of donor **118**. The mixture was filtered through Celite and then the Celite was washed with CH_2Cl_2. The CH_2Cl_2 solution was concentrated and the residue was purified with chromatographic column (4% acetone in toluene) to give product **128** (3.7 mg, 81%, recovered 0.8 mg of **54**, β/α > 50:1, R_f = 0.40, toluene-acetone, 18:1). ^1H NMR ($CDCl_3$): δ = 7.40-7.10 (m, 40 H, aromatics), 5.08 (d, 1 H, J = 7.8 Hz, H-1) and 4.36 (d, 1 H, J = 8.0 Hz, H-1'), 4.95, 4.80, 4.77, 4.68, and 4.67 (5 d, 6 H, 3 x O*CH*$_2$Ph), 4.46-4.30 (m, 8 H, 3 x O*CH*$_2$Ph, H-4' and H-4), 4.03 (dd, 1 H, J = 7.8, 9.8 Hz, H-2'), 3.98-3.96 (2 bd, 4 H, J = 13.5 Hz, 2 x N*CH*$_2$Ph), 3.95 (m, 1 H, H-6a), 3.60-3.32 (m, 9 H, 2 x H-6', H-6b, H-5, H-5', O*CH*$_2$(CH$_2$)$_6$, H-3', and H-3), 3.28 (dd, 1 H, J = 8.0, 10.0 Hz, H-2'), 1.65 (m, 2 H, OCH$_2$*CH*$_2$(CH$_2$)$_5$), 1.45-1.20 (m, 10 H, O(CH$_2$)$_2$(*CH*$_2$)$_5$CH$_3$), and 0.85 (t, 3 H, O(CH$_2$)$_7$*CH*$_3$); ^{13}C NMR ($CDCl_3$): δ = 140.77, 139.22, 138.78, 138.58, 138.14, 129.06, 128.49, 128.43, 128.34, 128.27, 128.23, 128.10, 128.05, 127.92, 127.83, 127.70, 127.62, 127.30, 127.16, 126.28, 103.86 and 100.88 (C-1', C-1), 82.52, 80.05, 79.47, 75.26, 74019, 73.84, 73.57, 73.16, 73.03, 72.79, 71.48, 69.84, 69.65, 68.93, 68.38, 60.00, 31.90, 30.23, 29.51, 29.34, 26.33, 22.71 and 14.13.

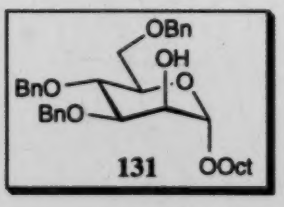
Octyl 4-O-(2-deoxy-2-amino-β-D-galactopyranosyl)-β-D-galactopyranoside (**129**).



129

A suspension of disaccharide **128** (5 mg), $Pd(OH)_2/C$ (10%, 5 mg) in EtOH (2 mL) with HCl (0.2%) was stirred under H_2 at rt for 3 h. TLC showed complete disappearance of starting material. The mixture was then filtered through a Millex-GV filter unit and then the filter unit was washed with methanol. The methanol solution was concentrated and the residue was purified using a C-18 Sep-Pak cartridge to give product **129** (1.7 mg, 92%, R_f = 0.46, CH_2Cl_2-MeOH-NH_4OH, 4:3:1). Selected physical data for **129**: ^1H NMR (D_2O): δ = 4.52 (d, 1 H, J = 8.2 Hz, H-1'), 4.41 (d, 1 H, J = 7.9 Hz, H-1) and 2.90 (dd, 1 H, H-2'); ^{13}C NMR (D_2O): δ = 103.50 ($J_{C1'-H1'}$ = 160.4 Hz, C-1), 105.50 ($J_{C1'-H1'}$ = 161.7 Hz, C-1'); HR-MS(ES): 454.2654 $[M+H]^+$.

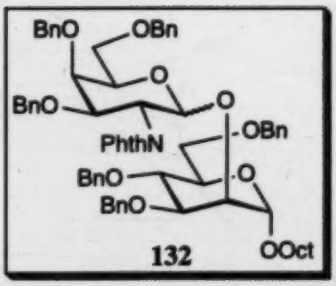
Octyl 3,4,6-tri-O-benzyl-α-D-mannopyranoside (**131**).



131

^1H NMR ($CDCl_3$): δ = 7.40-7.15 (m, 15 H, aromatics), 4.88 (d, 1 H, J = 2.6 Hz, H-1), 4.80 and 4.50 (2 d, 2 H, J = 11.0 Hz, O*CH*$_2$Ph), 4.71 and 4.66 (2 d, 2 H, J = 11.5 Hz, O*CH*$_2$Ph), 4.65 and 4.53 (2 d, 2 H, J = 12.5 Hz, O*CH*$_2$Ph), 4.02 (bdd, 1 H, J = 2.6, 4.0 Hz, H-2), 3.92-3.62 (m, 6 H, H-3, H-4, H-5, 2 x H-6, and O*CH*$(CH_2)_6$), 3.40 (m, 1 H, O*CH*$(CH_2)_6$), 1.60-1.50 (m, 2 H, $OCH_2CH_2(CH_2)_5$), 1.35-1.20 (bs, 10 H, $O(CH_2)_2(CH_2)_5CH_3$), and 0.85 (t, 3 H, $O(CH_2)_7CH_3$).

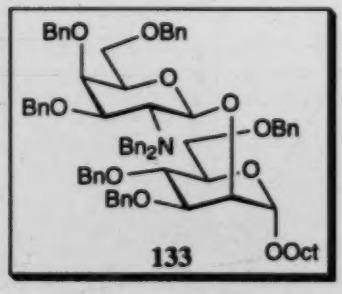
Octyl 2-O-(3,4,6-tri-O-benzyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl)-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**132**).



132

A mixture of glycosyl donor **116** (15 mg, 24.6 μmol), **131** (6.8 mg, 12.1 μmol) and 4 Å molecular sieves (200 mg) in CH_2Cl_2 (1 mL) was stirred for 1 h at rt, cooled to -30 °C and then $DMTSBF_4$ (9.5 mg, 48.4 μmol) was added under Ar. The temperature was increased to 0 °C slowly over 3 h. TLC showed complete disappearance of donor **116**. The mixture was filtered through Celite and then the Celite was washed with CH_2Cl_2. The CH_2Cl_2 solution was concentrated and the residue was purified with chromatographic column (hexane-EtOAc, 6:1) to give product **132** (8.8 mg, 75%, recovered 0.8 mg for **132**; β/α = 3:1, R_f = 0.45, hexane-EtOAc, 4:1). ^1H NMR ($CDCl_3$): δ = 7.70-7.00 (m, 34 H, aromatics), 5.23 (d, 1 H, J = 8.5 Hz, H-1'). 4.82 (dd, 1 H, J = 8.5, 11.5 Hz, H-2'), 4.50 (d, 1 H, J = 2.0 Hz, H-1), 5.00, 4.74, 4.73, 4.45, 4.61, 4.60, 4.44, 4.40, 4.33, 4.31, 4.13, and 4.03 (12 d, 12 H, 6 x OCH_2Ph), 4.33, 4.05, 3.80-3.40, and 3.20-3.03 (m, 13 H, 2 x H-6', 2 x H-6, H-5, H-5', $OCH_2(CH_2)_6$, H-3', H-3, H-4, H-4', and H-2), 1.40 (m, 2 H, $OCH_2CH_2(CH_2)_5$), 1.30-1.25 (m, 10 H, $O(CH_2)_2(CH_2)_5CH_3$), and 0.88 (t, 3 H, $O(CH_2)_7CH_3$).

Octyl 2-O-(3,4,6-tri-O-benzyl-2-deoxy-2-N,N-dibenzylamino-β-D-galactopyranosyl)-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**133**).

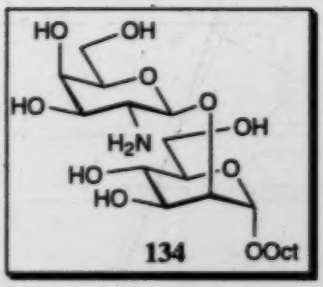


133

A mixture of glycosyl donor **118** (23 mg, 34.8 μmol), **131** (9.3 mg, 17.4 μmol) and 4 Å molecular sieves (200 mg) in CH_2Cl_2 (1 mL) was stirred for 1 h at rt, cooled to -30 °C and then $DMTSBF_4$ (13.6 mg, 69.6 μmol) was added under Ar. The temperature was increased to 0 °C slowly over 3 h. TLC showed

complete disappearance of donor **118**. The mixture was filtered through Celite and then the Celite was washed with CH_2Cl_2. The CH_2Cl_2 solution was concentrated and the residue was purified with chromatographic column (hexane-EtOAc, 12:1) to give product **133** (13.5 mg, 89%, recovered 2.0 mg of **131**, β/α > 11:1, R_f = 0.46, hexane-EtOAc, 4:1). Selected ^{1}H NMR ($CDCl_3$): δ = 7.50-7.10 (m, 40 H, aromatics), 5.03 (d, 1 H, J = 2.0 Hz, H-1) and 4.36 (d, 1 H, J = 7.8 Hz, H-1'), 1.60 (m, 2 H, $OCH_2CH_2(CH_2)_5$), 1.30 (m, 10 H, $O(CH_2)_2(CH_2)_5CH_3$), and 0.90 (t, 3 H, $O(CH_2)_7CH_3$); ^{13}C NMR ($CDCl_3$): δ = 140.42, 138.82, 138.42, 137.83, 129.03, 128.86, 128.75, 128.36, 128.20, 128.16, 128.01, 127.95, 127.83, 127.72, 127.57, 127.44, 127.31, 127.11, 127.02, 126.35, 101.40 (C-1'), 97.13 (C-1) and 59.05 (C-2'), 79.36, 77.95, 75.10, 74.34, 73.46, 72.81, 71.42, 70.33, 69.86, 68.88, 67.85, 59.05, 31.77, 29.56, 29.37, 29.17, 26.15, 22.60 and 14.03.

Octyl 2-O-(2-deoxy-2-amino-β-D-galactopyranosyl)-α-D-mannopyranoside (**134**).



A suspension of disaccharide **133** (13.5 mg), $Pd(OH)_2$/C (10%, 13 mg) in EtOH (2 mL) with HCl (0.2%) was stirred under H_2 at rt for 3 h. TLC showed complete disappearance of starting material. The mixture was then filtered through a Millex-GV filter unit and then the filter unit was washed with MeOH. The MeOH solution was concentrated and the residue was purified using a C-18 Sep-Pak cartridge to give product **134** (5.0 mg, 96%, R_f = 0.45, CH_2Cl_2-MeOH-NH_4OH, 4:3:1). Selected physical data for **134**: ^{1}H NMR (D_2O): δ = 4.98 (d, 1 H, J = 1.7 Hz, H-1), 4.38 (d, 1 H, J = 8.3 Hz, H-1') and 2.90 (dd, 1 H, H-2'); ^{13}C NMR (D_2O): δ = 102.80 ($J_{C1'-H1'}$ = 160.6 Hz, C-1'), 98.20 (J_{C1-H1} = 170.7 Hz, C-1); HR-MS(ES): 454.6257 $[M+H]^+$.

Chapter 6

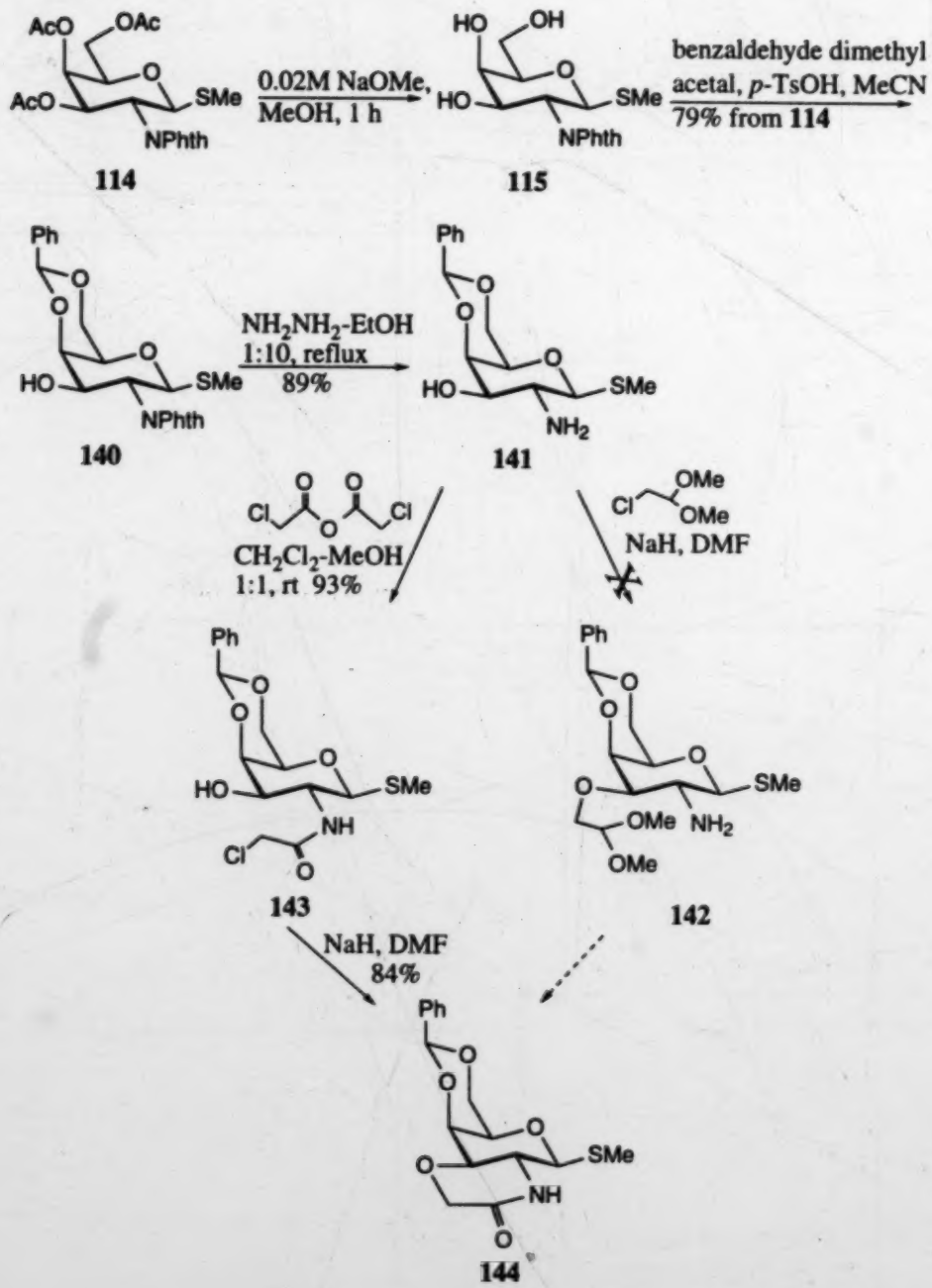
Studies Toward a New Method for 1,2-*cis*-Glycosylation with 2-Amino Sugars

6.1. Introduction.

6.1.1. Synthesis of Model Donors with a 2-*N*,3-*O* Linker.

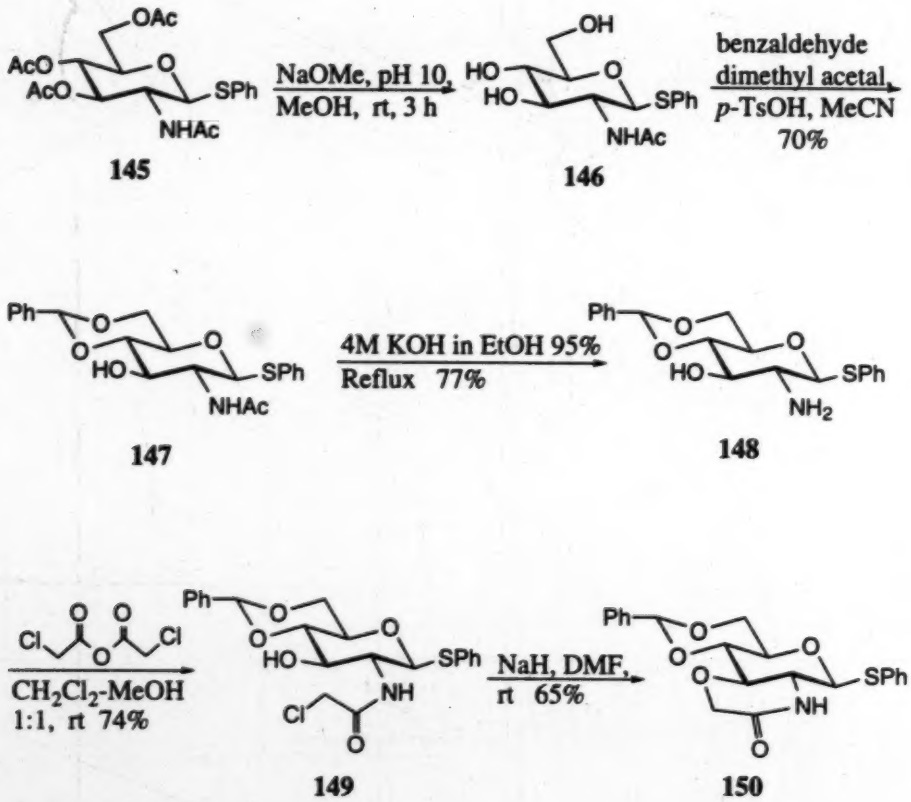
Scheme 5.5 outlined our design of a novel strategy for 1,2-*cis*-glycosylation of 2-amino sugars. We first synthesized 5 model donors with 3-*O*,2-*N* linkers. This involved the preparation of suitably protected 3-hydroxy-2-amino-thioglycoside donors followed by the construction of a linker between *O*-3 and *N*-2 as shown in Schemes 6.1 to 6.4.

The synthesis of the methyl 4,6-*O*-benzylidene-2,3-(*O*-methylenelactam)-1-thio-β-D-galactopyranoside donor (**144**) was performed as follows. Compounds **114** and **115** were prepared as described in Part I of this thesis. The benzylidenation of **115** with benzaldehyde dimethyl acetal in the presence of *p*-TsOH in MeCN gave **140** in 79% yield (from **144**) (Scheme 6.1). Hydrazinolysis of **140** in refluxing hydrazine-ethanol (1:10) for 3 h yielded the free amine product **141** (89%) [158]. Treatment of **141** with chloroacetaldehyde dimethyl acetal and NaH in DMF failed to give **142** [151]. Compound **141** did react, however, with chloroacetic anhydride in CH_2Cl_2-MeOH (1:1) at rt to afford



Scheme 6.1: Synthesis of the donor **144** with a 2,3-(*O*-methylenelactam)-linker.

143 (93%) which was then treated with NaH in DMF at rt to give the methyl 4,6-*O*-benzylidene-2,3-(*O*-methylenelactam)-1-thio-β-D-galactopyranosyl donor **144** (84%).

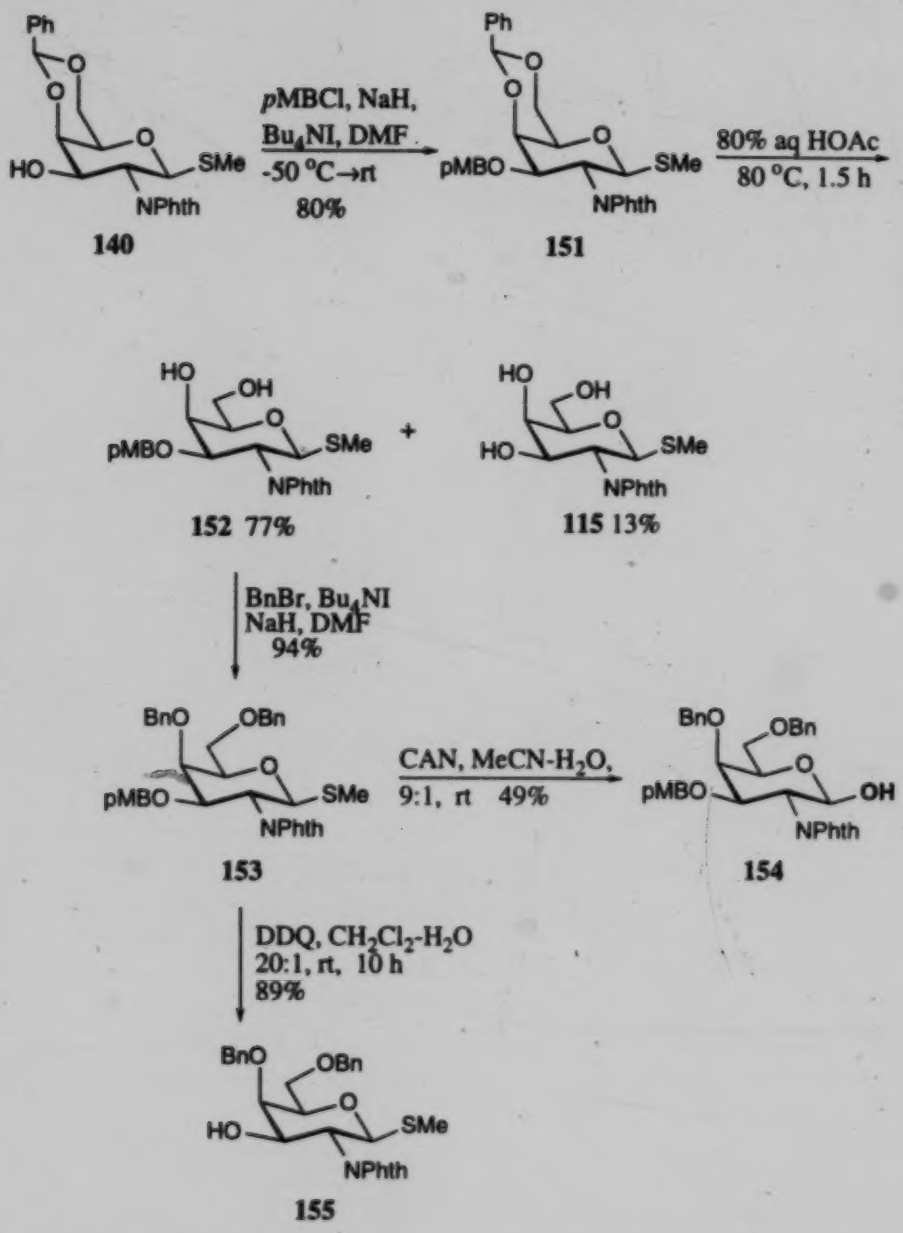


Scheme 6.2: Synthesis of the donor **150** with a 2,3-(*O*-methylenelactam)-linker.

We also prepared the phenyl 4,6-*O*-benzylidene-2,3-(*O*-methylenelactam)-1-thio-β-D-glucopyranosyl donor **150** to compare with donor **144**. The synthesis of **150** was performed as shown in Scheme 6.2. Deacetylation of compound **145**, which was prepared in this group by Dr. Carles Malet, with NaOMe in MeOH at pH 10 for 3 h gave **146**. Benzylidenation of **146** under standard conditions afforded product **147** in 70% yield (from **145**). *N*-Deacetylation of **147** with 4 M KOH in refluxing EtOH for 4 h

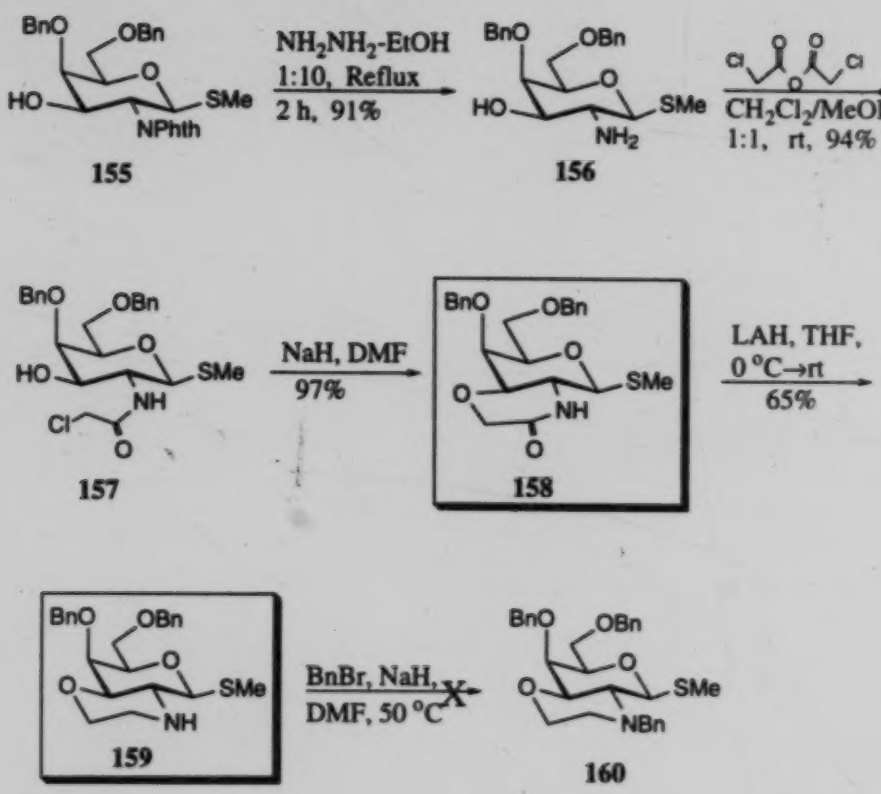
yielded the free amine **148** (77%) [153]. Treatment of **148** with chloroacetic anhydride in CH_2Cl_2-MeOH (1:1) gave **149** (74%). Finally, compound **149** was converted into the donor **150** in 65% yield by treatment with NaH in DMF at rt.

The 4,6-di-*O*-benzyl-β-D-galactopyranosyl donors with 2-*N*,3-*O*-linkers (**158-160**, and **165**) were also synthesized. Donors **158** and **159** were prepared as follows. Treatment of **140** with *p*-methoxybenzylchloride and NaH in the presence of Bu_4NI in DMF at -50 °C → rt gave **151** in 80% yield (Scheme 6.3). Debenzylidenation of **151** with 80% aq AcOH at 80 °C yielded the 4,6-diol **152** (77%) and the 3,4,6-triol **115** (13%). This was not a good method to remove the benzylidene because the 3-*O*-*p*-methoxybenzyl group was not stable under the conditions. Benzylation of **152** with benzyl bromide and NaH in the presence of Bu_4NI in DMF at 0 °C → rt gave **153** in 94% yield. Removal of the *p*-methoxybenzyl group with ceric (IV) ammonium nitrate (CAN) in acetonitrile-water (9:1) at rt produced only **154** (49%) [154]. Treatment of **153** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in CH_2Cl_2-H_2O (20:1) at 0 °C → rt overnight gave, however, the desired **155** in 89% yield (9% of starting material recovered) [155]. Hydrazinolysis of **155** in refluxing hydrazine-ethanol (1:10) for 2 h yielded the free amine product **156** (91%). Treatment of **156** with chloroacetic anhydride in CH_2Cl_2-MeOH (1:1) at rt afforded **157** (94% yield) which was then treated with NaH in DMF at rt to give the methyl 4,6-di-*O*-benzyl-2,3-(*O*-methylenelactam)-1-thio-β-D-galactopyranosyl donor **158** in 97% yield. Reaction of lactam **158** with Lawesson's reagent (2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide) in dry THF at rt failed to give any product [156]. However, compound **158** was reduced with lithium aluminum hydride (LAH) in refluxing dry THF to afford the methyl 4,6-di-*O*-benzyl-2-*N*-3-*O*-ethylene-1-thio-β-D-galactopyranoside donor (**159**) (65%) [152, 157]. Attempted *N*-benzylation of **159** with BnBr and NaH in DMF at rt, even at 50 °C overnight, did not result in any reaction. This led us to investigate a different route for the preparation of the donor **160** as shown below (Scheme 6.4).



Scheme 6.3: Synthesis of the 2-*N*,3-*O*-ethylene-linker donors **158** and **159**.

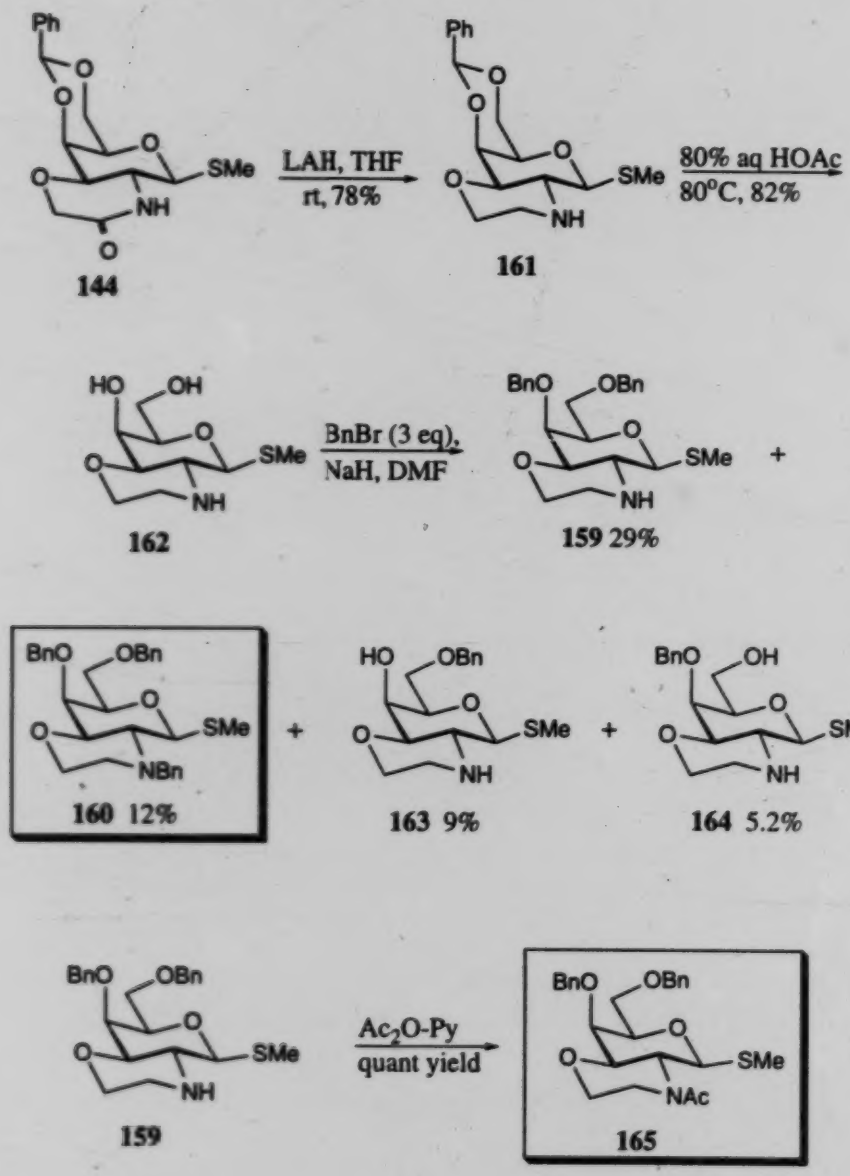
Reduction of compound **144** with LAH in THF at rt overnight and then at the boiling point for 1 h gave **161** (78%). Debenzylidenation of **161** using 80% aq AcOH at 80 °C afforded **162** in 82% yield. In order to synthesize **159** and **160** in one step,



Scheme 6.3: Synthesis of the 2-*N*,3-*O*-ethylene-linker donors **158** and**159** (contd).

compound **162** was benzylated with BnBr (3 equiv) and NaH (6 equiv) in DMF at 0 °C to rt overnight to give the 4,6-di-*O*-benzyl product **159** (29%), the 4,6-*O*-2-*N*-tribenzyl product **160** (12%), the 6-*O*-benzyl product **163** (9%) and the 4-*O*-benzyl product **164** (5%). When more BnBr and NaH were used, **160** was obtained as a major product. Compounds **163** and **164** could be retreated with BnBr and NaH in DMF to produce **159** and **160**. The position of the mono-*O*-benzyl group in **163** and **164** was confirmed by the ^{1}H NMR spectra of the *O*-acetylated compounds. We also attempted the selective *O*-benzylation of **162** to prepare the 4,6-di-*O*-benzyl product **159** using BaO (9 equiv), $Ba(OH)_2 \cdot H_2O$ (1 equiv) and BnBr (2 equiv) in DMF at rt for 2 d. TLC revealed the

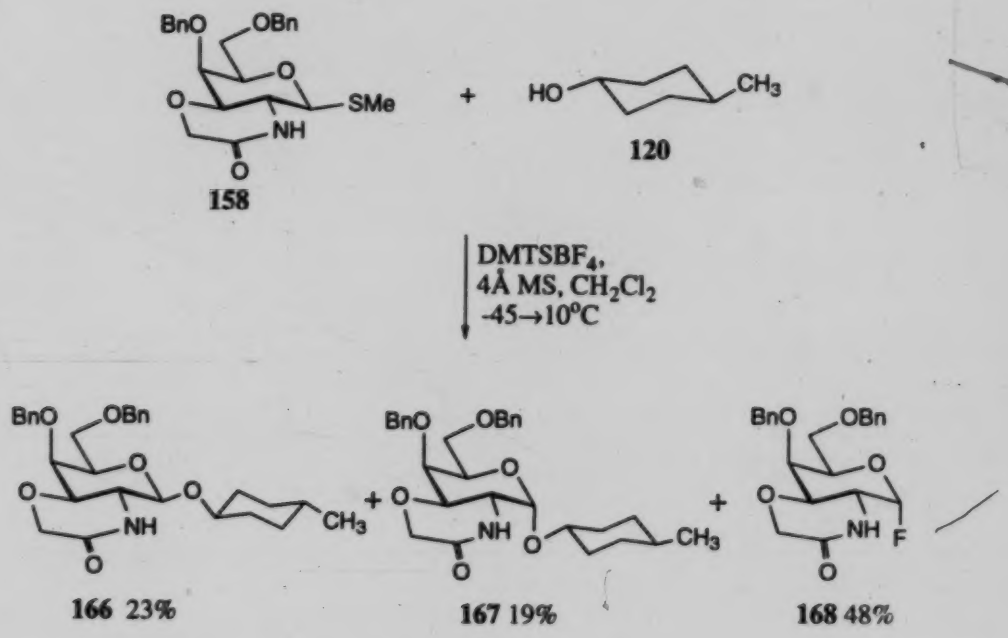
formation of a complex mixture. Finally, *N*-acetylation of **159** with Ac_2O-pyridine (1:2) gave the donor **165** in quantitative yield.



Scheme 6.4: Synthesis of the 2-*N*,3-*O*-ethylene-linker donor **160** and the 2-*N*-acetyl-2-*N*,3-*O*-ethylene-linker donor **165**.

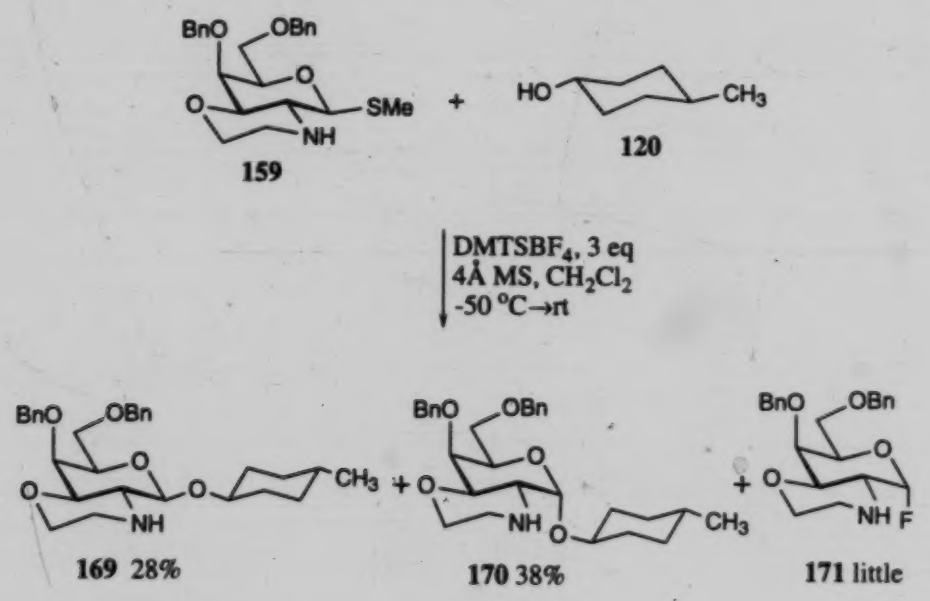
6.1.2. Glycosylation of Model Donors with *trans*-4-Methylcyclohexanol.

We next reacted the model donors **158-160** and **165** with a secondary alcohol in the presence of a neutral promotor to evaluate the stereoselectivity of the glycosylation reaction. Glycosylation of *trans*-4-methylcyclohexanol with the methyl 4,6-di-*O*-benzyl-2,3-(*O*-methylenelactam)-1-thio-β-D-galactopyranosyl donor **158** was promoted with $DMTSBF_4$ in the presence of 4 Å molecular sieves in CH_2Cl_2 at -45 → 10 °C for 3 h to give the β-linked product **166** (23%), the α-linked product **167** (19%) and the fluoride **168** (48%) (Scheme 6.5). The nucleophilicity of the fluoride ion from BF_4^- accounts for the formation of **168** as the major product.



Scheme 6.5: Glycosylation of alcohol **120** with donor **158**.

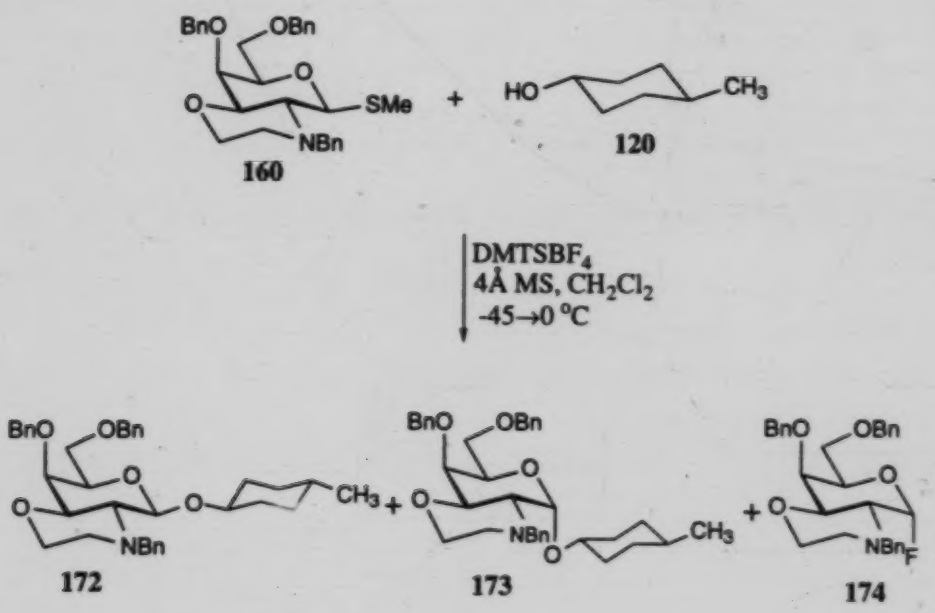
The methyl 4,6-di-*O*-benzyl-2-*N*-3-*O*-ethylene-1-thio-β-D-galactopyranosyl donor **159** was coupled with *trans*-4-methylcyclohexanol in the presence of $DMTSBF_4$ and 4 Å molecular sieves in CH_2Cl_2 at -50 °C → rt overnight to give the β-linked product **169** (28%), the α-linked product **170** (38%), and only traces of the fluoride product **171** (Scheme 6.6).



Scheme 6.6: Glycosylation of alcohol **120** with donor **159**.

trans-4-Methylcyclohexanol was glycosylated with the methyl 4,6-di-*O*-benzyl-2-*N*-benzyl-2-*N*-3-*O*-ethylene-1-thio-β-D-galactopyranosyl donor **160** in the presence of $DMTSBF_4$ and 4 Å molecular sieves in CH_2Cl_2 using different ratios of donor, acceptor and promotor at different temperatures, giving the β-linked product **172**, the α-linked product **173**, and the fluoride **174**. The results are summarized in Scheme 6.7. When additional acceptor and less promotor were used at a low temperature, more α-linked

product was obtained. The best α:β ratio obtained was 6:1 when the reagents used were **160**:alcohol:$DMTSBF_4$ 1:6:2.

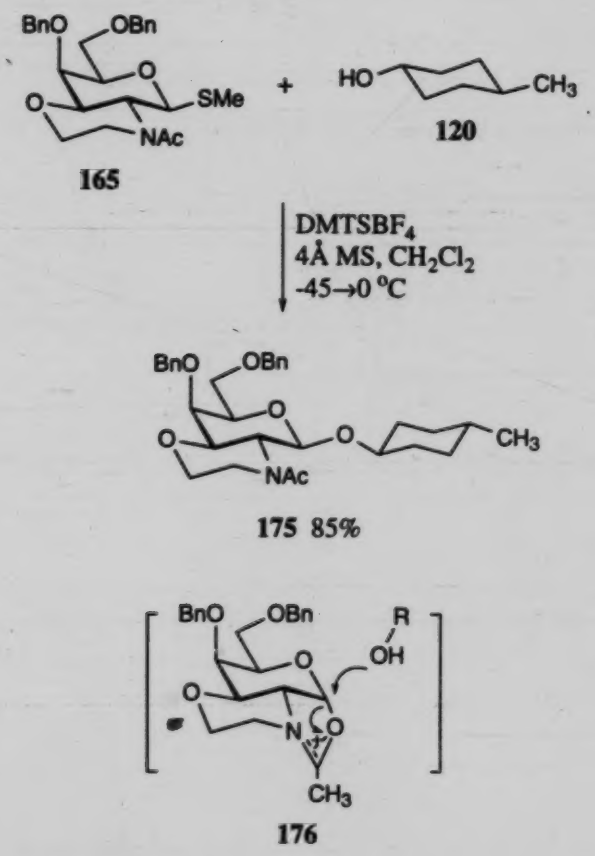


	ratio				
160	1	1	1	1	
Alcohol	2	6	6	2	
$DMTSBF_4$	2	6	2	2	-20→10 °C
172:173:174	3:6:9	3:10:9	3:19:14	4:9:14	

Scheme 6.7: Glycosylation of alcohol **120** with donor **160**.

Coupling of the methyl 2-*N*-acetyl-4,6-di-*O*-benzyl-2-*N*-3-*O*-ethylene-1-thio-β-D-galactopyranosyl donor **165** with *trans*-4-methylcyclohexanol in the presence of $DMTSBF_4$ and 4 Å molecular sieves in CH_2Cl_2 at -45 → 0 °C overnight gave only the β-linked product **175** in high yield (85%) (Scheme 6.8). We believe that the intermediate **176** (Scheme 6.8) is responsible for the stereoselectivity in this reaction. The intermediate

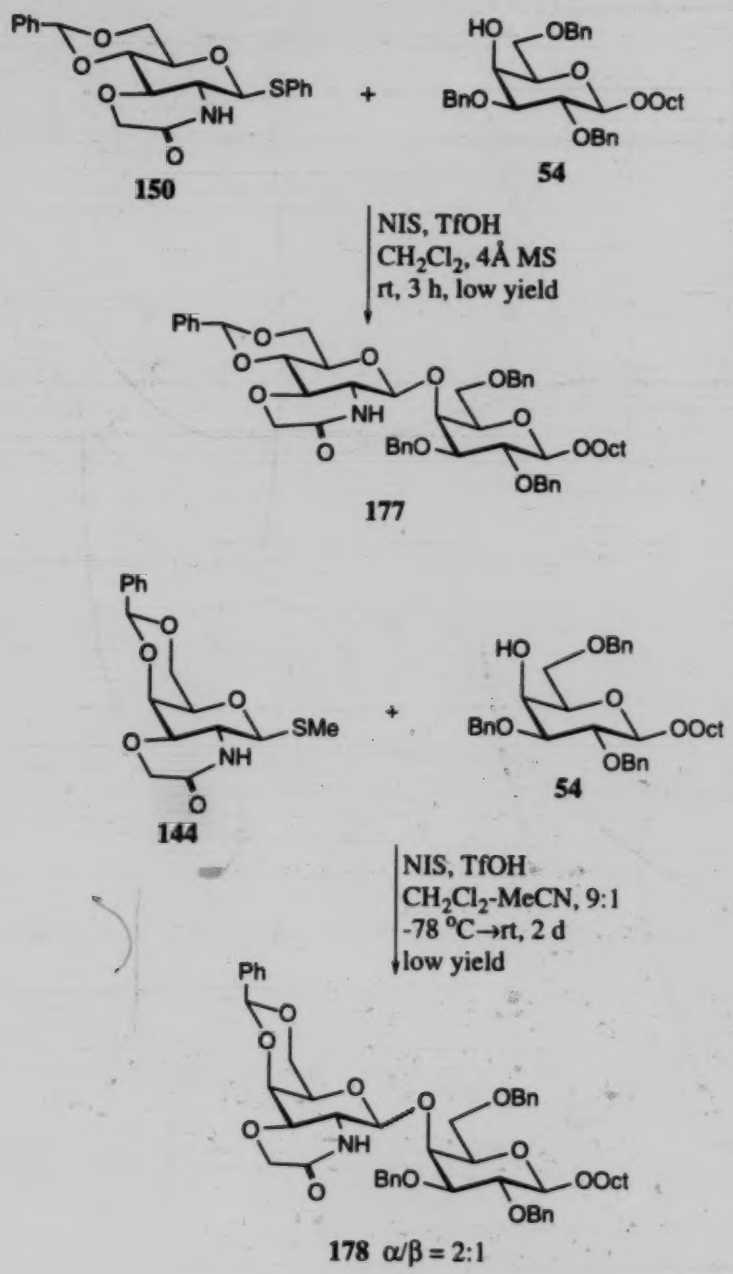
176 was stable enough to be detected by TLC and it also persisted for several hours at the reaction temperature.



Scheme 6.8: Glycosylation of alcohol **120** with donor **165.**

6.1.3. Glycosylation of Sugar Alcohols with Model Donors.

We reacted the two different model donors, **150** and **144**, with octyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (**54**) and octyl 2-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**42**) acceptors, respectively, as shown in Schemes 6.9 and 6.10.

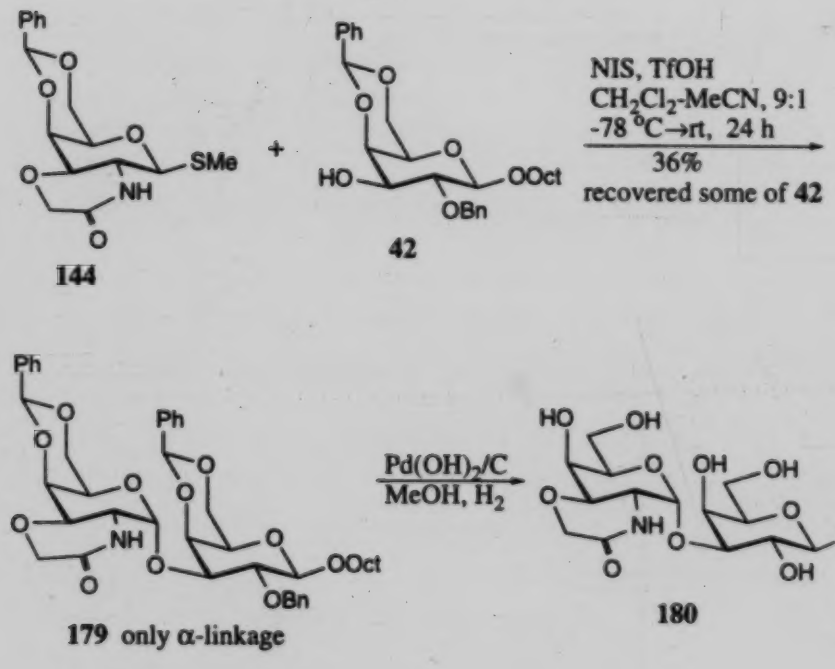


Scheme 6.9: Glycosylation of alcohol **54** with donors **150** and **144**.

Donor **150** was coupled with acceptor **54** using NIS and TfOH as promoters in the presence of 4 Å molecular sieves in CH_2Cl_2 at -78 °C → rt for 3 h to give only the β-

linked disaccharide **177** in low yield (about 20%) (Scheme 6.9). This poor result can probably be attributed to the thiophenyl donor which was relatively unreactive. However, when acceptor **54** was glycosylated with donor **144** using NIS and TfOH as promoters in the presence of 4 Å molecular sieves in CH_2Cl_2-MeCN (9:1) at -78 °C → rt for 2 d, a mixture of disaccharides **178** ($\alpha/\beta > 2:1$) was obtained in low yield (about 30%) (Scheme 6.9).

Coupling of **144** with the relatively more reactive acceptor, octyl 2-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**42**), promoted by NIS and TfOH in the presence of 4 Å molecular sieves in CH_2Cl_2-MeCN (9:1) at -78 °C → rt for 1 d gave only the α-linked disaccharide **179** in 36% yield (Scheme 6.10). Hydrogenolysis of **179** using Pearlman's catalyst ($Pd(OH)_2/C$) in MeOH quantitatively afforded the disaccharide **180**.



Scheme 6.10: Glycosylation of **42** with donor **144**.

In summary, from the initial results obtained in the reactions described herein, glycosylation of acceptors (alcohol and sugars) with the model 2,3-tethered donors proceeded with high α-stereoselectivity when neighboring participation in suppressed. Futher investigation of such tethered compounds will require the preparation of a donor with removable 2-*N*,3-*O* linker, such as a 1,2-diphenylethylene linker.

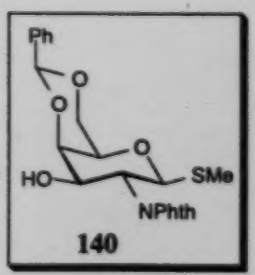
6.2. Experimental Section.

6.2.1. General Methods.

General methods were the same as described in Chapter 2, Part I. Compounds **114**, **54** and **42** were synthesized in Part I and **145** was synthesized in this group by Dr. Carles Malet.

6.2.2. Experimental.

Methyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside (**140**).

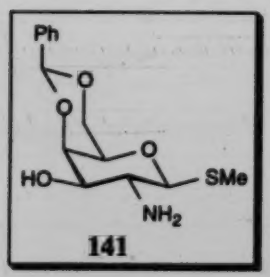


140

Crude product **115** (400 mg, 1.18 mmol) and benzaldehyde dimethyl acetal (0.355 mL, 2.36 mmol) were dissolved in MeCN (50 mL) at rt, followed by addition of *p*-TsOH (catalytic amount). After the mixture was stirred for 3 h, TLC (R_f = 0.45, hexane-EtOAc, 1:1) indicated the absence of starting material. The solution was neutralized with Et_3N and then concentrated. The resulting residue was subjected to column chromatography (hexane-EtOAc, 1.5:1) to give product **140** (400 mg) in 79% from **114**. ^1H NMR ($CDCl_3$): δ = 7.90-7.35 (m, 9 H,

aromatics), 5.52 (s, 1 H, Ph*CH*O_2), 5.28 (d, 1 H, J = 10.0 Hz, H-1), 4.62 (ddd, 1 H, J = 10.0, 10.0, 3.4 Hz, H-3), 4.56 (dd, 1 H, J = 10.0, 10.0 Hz, H-2), 4.42 (dd, 1 H, J = 12.5, 1.5 Hz, H-6a), 4.34 (dd, 1 H, J = 3.5, 1.5 Hz, H-4), 4.16 (dd, 1 H, J = 12.5, 1.8 Hz, H-6b), 3.55 (td, 1 H, J = 1.8, 1.5 Hz, H-5), 2.51 (d, 1 H, J = 10.0 Hz, OH), and 2.25 (s, 3 H, SMe).

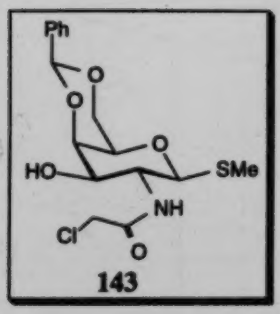
Methyl 2-amino-4,6-O-benzylidene-2-deoxy-1-thio-β-D-galactopyranoside (**141**).



141

A solution of compound **140** (350 mg, 0.82 mmol) in hydrazine monohydrate-ethanol (1:1, 40 mL) was refluxed for 3 h and then concentrated. The resulting residue was co-evaporated with ethanol (3 x 20 mL) and then with toluene (2 x 20 mL). The crude product was purified with silica gel column (15:1, CH_2Cl_2-MeOH containing 1% of Et_3N) to afford **141** (240 mg, 89%; R_f = 0.25, CH_2Cl_2-MeOH, 10:1, containing 1% of Et_3N). ^{1}H NMR ($CDCl_3$): δ = 7.52-7.34 (m, 5 H, aromatic), 5.56 (s, 1 H, Ph*CH*O_2), 4.35 (dd, 1 H, J = 12.5, 1.5 Hz, H-6a), 4.19 (dd, 1 H, J = 3.5, 1.5 Hz, H-4), 4.18 (d, 1 H, J = 9.5 Hz, H-1), 4.03 (dd, 1 H, J = 12.5, 1.8 Hz, H-6b), 3.55-3.50 (m, 2 H, H-5 and H-3), 3.15 (dd, 1 H, J = 9.5, 9.5 Hz, H-2), and 2.22 (s, 3 H, SMe).

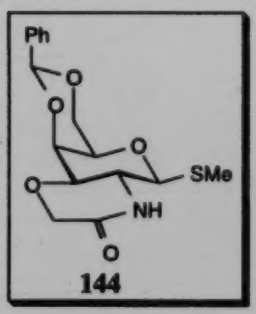
Methyl 4,6-O-benzylidene-2-chloroacetimido-2-deoxy-1-thio-β-D-galactopyranoside (**143**).



143

To a solution of compound **141** (20 mg, 61 μmol) in CH_2Cl_2-MeOH (1:1, 2 mL) was added chloroacetic anhydride (15.6 mg, 91 μmol) at rt. After the mixture was stirred for 5 h, TLC (R_f = 0.45, CH_2Cl_2-MeOH, 10:1) indicated the absence of starting material. The solution was diluted with CH_2Cl_2 (50 mL) and washed with Brine (2 x 30 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated. The resulting residue was subjected to column chromatography (CH_2Cl_2-MeOH, 20:1) to give **143** (22 mg, 93%). ^{1}H NMR ($CDCl_3$): δ = 7.52-7.35 (m, 5 H, aromatic), 6.58 (bd, 1 H, J = 8.5 Hz, *NH*), 5.59 (s, 1 H, Ph*CH*O_2), 4.54 (d, 1 H, J = 10.0 Hz, H-1), 4.38 (dd, 1 H, J = 12.5, 1.5 Hz, H-6a), 4.27 (dd, 1 H, J = 3.5, 1.0 Hz, H-4), 4.20 (ddd, 1 H, J = 10.0, 10.0, 8.5 Hz, H-2), 4.11 (s, 2 H, Cl*CH*$_2$CO), 4.06 (dd, 1 H, J = 12.5, 1.8 Hz, H-6b), 3.87 (bddd, 1 H, J = 10.0, 8.5, 3.5 Hz, H-3), 3.57 (dd, 1 H, J = 1.5, 1.8 Hz, H-5), 2.85 (d, 1 H, J = 8.5 Hz, *OH*), and 2.22 (s, 3 H, SMe). ^{13}C NMR (DMSO-D_6): δ = 166.03, 138.67, 128.81, 128.05, 126.40, 99.99, 83.23, 75.34, 70.33, 69.37, 68.73, 50.29, 42.93, 10.97.

Methyl 4,6-O-benzylidene-2,3-(3-O-methylenelactam)-2-deoxy-1-thio-β-D-galactopyranoside (**144**).

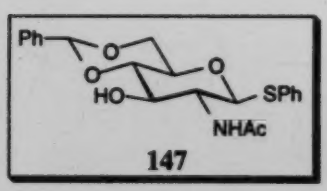


144

To a solution of compound **143** (33 mg, 85 μmol) in DMF (5 mL), NaH (5 mg, 170 μmol, 80% in oil) was added at 0 °C with stirring. The suspension was slowly increased to rt. After the mixture was stirred overnight, some MeOH was added to the reaction solution to decompose the excess of NaH and then diluted with EtOAc (30 mL). The solution was washed with Brine (3 x 20 mL), dried with $MgSO_4$, filtered, and then concentrated. The resulting

residue was subjected to column chromatography (toluene-acetone, 2:1) to give **144** (25 mg, 84%). ^{1}H NMR ($CDCl_3$): δ = 7.50-7.15 (m, 5 H, aromatic), 6.25 (bs, 1 H, *NH*), 5.58 (s, 1 H, Ph*CH*O_2), 4.42 and 4.25 (2 d, 2 H, J = 17.0 Hz, O*CH*$_2$CON), 4.30 (d, 1 H, J = 10.0 Hz, H-1), 4.39 (dd, 1 H, J = 12.5, 1.5 Hz, H-6a), 4.40 (d, 1 H, J = 3.5 Hz, H-4), 4.10 (dd, 1 H, J = 12.5, 1.8 Hz, H-6b), 4.97 (dd, 1 H, J = 10.0, 10.0 Hz, H-2), 3.58 (dd, 1 H, J = 10.0, 3.5 Hz, H-3), 3.60 (bs, 1 H, H-5), and 2.22 (s, 3 H, SMe). ^{13}C NMR ($CDCl_3$): δ = 168.17, 137.36, 129.41, 128.39, 126.49, 101.45, 82.71, 76.83, 72.87, 70.31, 69.55, 68.32, 48.66, 10.19.

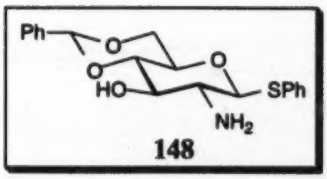
Phenyl 2-acetamido-4,6-O-benzylidene-2-deoxy-1-thio-β-D-glucopyranoside (**147**).



147

Compound **146** (700 mg, 2.24 mmol) and benzaldehyde dimethyl acetal (0.67 mL, 4.48 mmol) were dissolved in MeCN (100 mL) at rt and then *p*-TsOH (catalytic amount) was added. After the mixture was stirred for 3 h, TLC (R_f = 0.67, CH_2Cl_2-MeOH, 6:1) indicated the absence of starting material. The solution was neutralized with Et_3N and then concentrated. The resulting residue was recrystalized with 100% ethanol to give product **147** (625 mg, 79%). ^{1}H NMR ($CDCl_3$): δ = 7.50-7.25 (m, 10 H, aromatics), 5.52 (s, 1 H, Ph*CH*O_2), 5.09 (d, 1 H, J = 10.0 Hz, H-1), 4.75 (d, 1 H, J = 4.5 Hz, *NH*), 4.24 (dd, 1 H, J = 10.0, 4.5 Hz, H-6e), 3.95-3.90 (m, 1 H, H-5), 3.87 (dd, 1 H, J = 8.5, 10.0 Hz, H-4), 4.76 (dd, 1 H, J = 10.0, 10.0 Hz, H-3), 3.60-3.53 (m, 1 H, H-6a), 3.52 (ddd, 1 H, J = 10.0, 10.0, 4.5 Hz, H-2), 1.8 (s, 3 H, NAc).

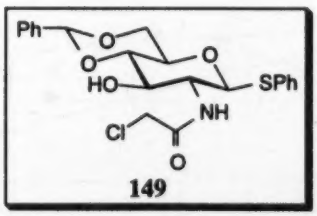
Phenyl 2-amino-4,6-O-benzylidene-2-deoxy-1-thio-β-D-glucopyranoside (**148**).



148

A solution of compound **147** (1.3 g, 3.24 mmol) in KOH in 95% ethanol (4 M, 60 mL) was refluxed for 4 h. TLC (detected by ninhydrine [168], R_f = 0.42, CH_2Cl_2-MeOH, 10:1, containing 1% of aq ammonia) indicated the absence of starting material. The solution was cooled to rt, diluted with CH_2Cl_2 (200 mL) and then washed with Brine (2 x 150 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated. The solid residue was recrystalized with EtOAc-hexane to give product **148** (900 mg, 77%). ^{1}H NMR ($CDCl_3$): δ = 7.60-7.25 (m, 10 H, aromatics), 5.51 (s, 1 H, Ph*CH*O_2), 4.61 (d, 1 H, J = 10.0 Hz, H-1), 4.34 (dd, 1 H, J = 10.0, 4.5 Hz, H-6e), 3.70 (dd, 1 H, J = 9.0, 9.0 Hz, H-4), 3.78 (dd, 1 H, J = 10.0, 10.0 Hz, H-6a), 3.52-3.49 (m, 2 H, H-3 and H-5), and 2.82 (dd, 1 H, J = 10.0, 10.0 Hz, H-2).

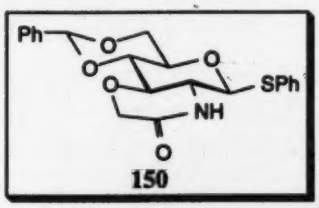
Phenyl 4,6-O-benzylidene-2-chloroacetamido-2-deoxy-1-thio-β-D-glucopyranoside (**149**).



149

To a solution of compound **148** (20 mg, 56 μmol) in CH_2Cl_2-MeOH (1:1, 2 mL) was added chloroacetic anhydride (14 mg, 84 μmol) at rt. After the mixture was stirred overnight, TLC (R_f = 0.43, CH_2Cl_2-MeOH, 20:1) indicated the absence of starting material. The solution was diluted with CH_2Cl_2 (50 mL) and washed with Brine (2 x 30 mL). The organic layer was dried with $MgSO_4$, filtered, and then concentrated. The resulting residue was subjected to column chromatography (toluene-acetone, 1.5:1) to give white powder **149** (21 mg, 74%). ^{1}H NMR ($CDCl_3$): δ = 7.60-7.30 (m, 10 H, aromatics), 6.86 (bd, 1 H, J = 8.5 Hz, *NH*), 5.56 (s, 1 H, Ph*CH*O_2), 5.03 (d, 1 H, J = 10.0 Hz, H-1), 4.39 (dd, 1 H, J = 10.5, 4.5 Hz, H-6e), 4.15 (dd, 1 H, J = 9.0, 9.0 Hz, H-4), 4.12 (s, 2 H, O*CH*$_2$CON), 3.84-3.50 (m, 4 H, H-6a, H-5, H-2 and H-3).

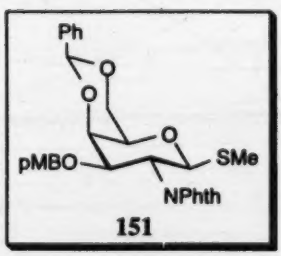
Phenyl 4,6-O-benzylidene-2,3-(3-O-methylenelactam)-2-deoxy-1-thio-β-D-glucopyranoside (**150**).



150

To a solution of compound **149** (20 mg, 46 μmol) in DMF (3 mL), NaH (2.8 mg, 92 μmol, 80% in oil) was added at 0 °C. The suspension was slowly increased to rt. After the mixture was stirred overnight, MeOH was added to the reaction solution to decompose the excess of NaH and then diluted with EtOAc (30 mL). The solution was washed with Brine (3 x 20 mL), dried with $MgSO_4$, filtered, and then concentrated. The resulting residue was subjected to column chromatography (toluene-acetone, 3:1) to give **150** (12 mg, 65%; R_f = 0.65, toluene-acetone, 3:2). ^1H NMR ($CDCl_3$): δ = 7.60-7.30 (m, 10 H, aromatics), 6.40 (s, 1 H, *NH*), 5.55 (s, 1 H, Ph*CHO*$_2$), 4.60 (d, 1 H, J = 10.0 Hz, H-1), 4.42 (dd, 1 H, J = 11.5, 4.5 Hz, H-6e), 4.35 and 4.24 (2 d, 2 H, J = 17.0 Hz, O*CH*$_2$CON), 3.85 (dd, 1 H, J = 11.5, 11.5 Hz, H-6a), 4.74-3.70 (m, 2 H, H-4 and H-3), 3.66-3.58 (m, 1 H, H-5), 3.32-3.24 (m, 1 H, H-2). ^{13}C NMR ($CDCl_3$): δ = 168.24, 136.54, 133.86, 129.40, 129.27, 129.06, 128.40, 126.35, 102.14, 85.92, 78.03, 77.30, 71.94, 68.45, 68.03, 54.72.

Methyl 4,6-O-benzylidene-2-deoxy-3-(p-methoxybenzyl)-2-phthalimido-1-thio-β-D-galactopyranoside (**151**).

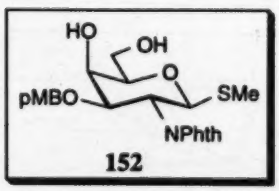


151

Compound **140** (1.52 g, 3.65 mmol), NaH (128 mg, 4.27 mmol, 80% in oil), and Bu_4NI (1.58 g, 4.27 mmol) in DMF (16 mL) was stirred at -45 °C for 30 min, followed by addition of *p*-methoxybenzyl chloride (0.49 mL, 3.61 mmol). The mixture was allowed to reach rt. Stirring was continued

for 1 h, during which time, one more addition of *p*-methoxybenzyl chloride (0.1 mL, 0.73 mmol) was made. After 2 h, 0.6 equiv of each reagent were added. After 2 h more, MeOH was added to the reaction mixture to decompose the excess of NaH and then the mixture was diluted with EtOAc (200 mL), washed with brine (2 x 100 mL), dried with $MgSO_4$, filtered, and concentrated. The resulting residue was applied to column of silica gel (hexane-EtOAc, 1:1) to give pure product **151** (1.56 g, 80%; R_f = 0.36, hexane-EtOAc, 1:1; and [α] = + 55.0°, *c* = 1.3, $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 7.90-7.30 (m, 9 H, aromatics), 7.02 and 6.56 (2 d, 4 H, aromatic of *p*MB), 5.52 (s, 1 H, Ph*CH*O_2), 5.28 (d, 1 H, J = 10.5 Hz, H-1), 4.82 (dd, 1 H, J = 10.5, 10.5 Hz, H-2), 4.55 and 4.37 (2 d, 2 H, J = 12.5 Hz, *p*MB), 4.47 (dd, 1 H, J = 10.5, 3.4 Hz, H-3), 4.35 (dd, 1 H, J = 12.5, 1.5 Hz, H-6a), 4.25 (d, 1 H, J = 3.5 Hz, H-4), 4.15 (dd, 1 H, J = 12.5, 1.5 Hz, H-6b), 3.68 (s, 3 H, OMe), 3.59 (bs, 1 H, H-5), and 2.23 (s, 3 H, SMe). ^{13}C NMR ($CDCl_3$): δ = 168.43, 167.70, 159.12, 137.96, 134.02, 133.89, 131.86, 131.65, 130.01, 129.25, 129.13, 128.31, 126.57, 123.61, 123.08, 113.58, 101.40, 79.70, 74.39, 73.04, 70.88, 70.13, 69.61, 55.12, 50.04, 10.23.

Methyl 2-deoxy-3-(p-methoxybenzyl)-2-phthalimido-1-thio-β-D-galactopyranoside (**152**).

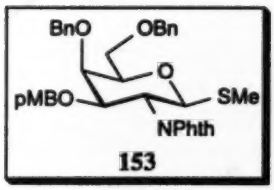


152

A solution of compound **151** (900 mg) in aq HOAc (80%, 100 mL) was heated at 80 °C with stirring for 1.5 h and concentrated in vacuo, co-concentrated with toluene (2 x 50mL). The residue was applied to a silica gel column (toluene-acetone, 2:1) to afford **152** (580 mg, 77%; R_f = 0.37, CH_2Cl_2-MeOH, 15:1). ^1H NMR ($CDCl_3$): δ = 7.90-7.60 (m, 4 H, aromatic), 6.95 and 6.48 (2 d, 4 H, aromatic of *p*MB), 5.13 (d, 1 H, J = 10.5 Hz, H-1), 4.54 (dd, 1 H, J = 10.5, 10.5 Hz, H-2), 4.55 and 4.26 (2 d, 2 H, J = 12.5 Hz, *p*MB), 4.32 (dd, 1 H, J = 10.5, 3.4 Hz, H-3), 4.15 (d, 1 H, J = 3.5 Hz, H-4), 4.06-3.98 (m, 1 H, H-6a), 3.90-3.81 (m, 1 H, H-6b), 3.73 (m, 1

H, H-5), 3.66 (s, 3 H, OMe), and 2.12 (s, 3 H, SMe). ^{13}C NMR ($CDCl_3$): δ = 168.16, 167.76, 159.30, 133.96, 133.90, 131.77, 131.59, 129.57, 129.16, 123.53, 123.14, 113.72, 80.49, 78.31, 75.11, 71.20, 66.59, 62.93, 55.03, 50.32, 20.97, 10.99.

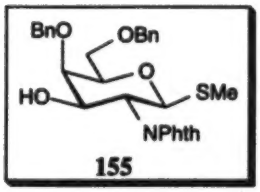
Methyl 4,6-di-O-benzyl-2-deoxy-3-(p-methoxybenzyl)-2-phthalimido-1-thio-β-D-galactopyranoside (**153**).



153

Compound **152** (520 mg, 1.13 mmol), NaH (166 mg, 5.53 mmol, 80% in oil), and Bu_4NI (1.67 g, 5.53 mmol) in DMF (10 mL) was stirred at 0 °C for 30 min and then BnBr (0.66 mL, 5.53 mmol) was added to the reaction solution. The mixture was allowed to reach rt. Stirring was continued for 4 h during which time one more addition of BnBr (0.4 mL) and NaH (100 mg) was made. MeOH was added to the reaction mixture to decompose the excess of NaH. The solution was diluted with EtOAc (200 mL), washed with Brine (2 x 100 mL), dried with $MgSO_4$, filtered, and concentrated. The resulting residue was applied to column of silica gel (hexane-EtOAc, 5:2) to give pure product **153** (680 mg, 94%; R_f = 0.35, hexane-EtOAc, 2:1). ^1H NMR ($CDCl_3$): δ = 7.90-7.25 (m, 14 H, aromatics), 6.95 and 6.50 (2 d, 4 H, aromatic of *p*MB), 5.15 (d, 1 H, J = 10.5 Hz, H-1), 4.78 (dd, 1 H, J = 10.5, 10.5 Hz, H-2), 5.00 and 4.61 (2 d, 2 H, J = 11.5 Hz, PhCH_2O), 4.54 and 4.24 (2 d, 2 H, J = 11.5 Hz, PhCH_2O), 4.54 and 4.24 (2 d, 2 H, J = 12.5 Hz, *p*MB), 4.36 (dd, 1 H, J = 10.5, 2.5 Hz, H-3), 4.07 (d, 1 H, J = 2.5 Hz, H-4), 3.81 (bt, 1 H, J = 6.5 Hz, H-5), 3.39 (s, 3 H, OMe), 3.66 (bd, 2 H, J = 6.5 Hz, H-6a and H-6b), and 2.15 (s, 3 H, SMe). ^{13}C NMR ($CDCl_3$): δ = 168.86, 137.99, 133.87, 129.92, 129.31, 128.50, 128.27, 127.99, 127.86, 127.51, 123.08, 113.61, 80.75, 77.17, 74.57, 73.58, 72.50, 71.20, 68.58, 55.10, 51.10, 11.09.

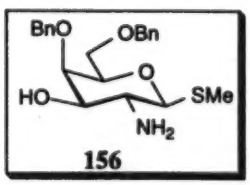
Methyl 4,6-di-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside (**155**).



155

DDQ (283 mg, 1.22 mmol) was added to a stirred solution of **153** (650 mg, 1.17 mmol) in CH_2Cl_2 (15 mL) saturated with water at rt. After stirring overnight, the reaction was complete, and the organic layer was successively washed with satd $NaHCO_3$ and water, dried with $MgSO_4$, filtered and concentrated. Column chromatography (hexane-EtOAc, 2:1) gave product **155** (470 mg, 89%; R_f = 0.27, hexane-EtOAc, 2:1). ^1H NMR ($CDCl_3$): δ = 7.85-7.60 (m, 14 H, aromatics), 5.20 (1 H, d, *J* 10.5 Hz, and t, *J* 12.0 Hz, H-1; "Virtual long-rang coupling of the anomeric proton of carbohydrates", *Carbohydr. Res.* 125(1984), 161-164), 4.80 and 4.62 (2 d, 2 H, J = 12.0 Hz, PhCH_2O), 4.59 and 4.53 (2 d, 2 H, J = 12.0 Hz, PhCH_2O), 4.42 (d, 2 H, J = 7.0 Hz, H-2 and H-3), 4.04 (d, 1 H, J = 1.5 Hz, H-4), 3.81 (ddd, 1 H, J = 6.5, 6.0, 0.8 Hz, H-5), 3.76-3.70 (m, 2 H, H-6a and H-6b), and 2.16 (s, 3 H, SMe). ^{13}C NMR ($CDCl_3$): δ = 168.46, 140.73, 138.12, 137.70, 134.11, 128.77, 128.56, 128.14, 127.99, 127.88, 123.63, 123.29, 103.22, 80.91, 77.37, 76.29, 75.34, 73.60, 69.24, 67.96, 53.57, 30.91, 11.41.

Methyl 2-amino-4,6-di-O-benzyl-2-deoxy-1-thio-β-D-galactopyranoside (**156**).

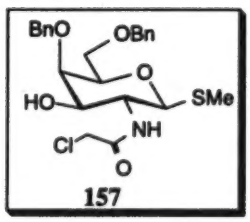


156

A solution of compound **155** (460 mg, 0.87 mmol) in hydrazine monohydrate-ethanol (1:1, 55 mL) was refluxed for 2 h and then concentrated. The resulting residue was co-evaporated with ethanol (3 x 50 mL) and then with toluene (2 x 50 mL). The crude product was purified with silica gel column (2:1, toluene-acetone containing 1% of Et_3N) to afford **156** (315 mg, 91%; R_f = 0.15, 2:1, toluene-acetone containing 1% of

Et_3N). ^1H NMR ($CDCl_3$): δ = 7.40-7.25 (m, 10 H, aromatics), 4.73 and 4.67 (2 d, 2 H, J = 12.0 Hz, Ph*CH*$_2$O), 4.54 and 4.48 (2 d, 2 H, J = 12.0 Hz, Ph*CH*$_2$O), 4.12 (d, 1 H, J = 9.8 Hz, H-1), 3.88 (d, 1 H, J = 3.5 Hz, H-4), 3.72-3.64 (m, 3 H, H-5, H-6a and H-6b), 3.40 (dd, 1 H, J = 9.8, 3.5 Hz, H-3), 2.97 (dd, 1 H, J = 9.8, 9.8 Hz, H-2), and 2.20 (s, 3 H, SMe). ^{13}C NMR ($CDCl_3$): δ = 138.53, 137.81, 128.56, 128.51, 127.96, 127.91, 87.96, 77.40, 75.56, 75.16, 73.61, 68.55, 53.52, 12.29.

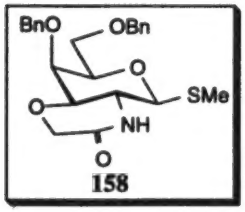
Methyl 4,6-di-O-benzyl-2-chloroacetamido-2-deoxy-1-thio-β-D-galactopyranoside (**157**).



157

To a solution of compound **156** (140 mg, 36 μmol) in CH_2Cl_2-MeOH (1:1, 26 mL) was added chloroacetic anhydride (93 mg, 54 μmol) at rt. After the mixture was stirred overnight, TLC (R_f = 0.26, toluene-acetone, 3:1) indicated the absence of starting material. The solution was diluted with CH_2Cl_2 (100 mL) and washed with Brine (2 x 50 mL). The organic layer was dried with $MgSO_4$, filtered, and then concentrated. The resulting residue was subjected to column chromatography (toluene-acetone, 3:1) to give **157** (145 mg, 92%; [α] = -9°, *c* = 0.3, $CHCl_3$). ^1H NMR ($CDCl_3$): δ = 7.40-7.27 (m, 10 H, aromatics), 6.58 (d, 1 H, J = 8.0 Hz, *NH*), 4.75 and 4.71 (2 d, 2 H, J = 11.5.0 Hz, Ph*CH*$_2$O), 4.52 and 4.47 (2 d, 2 H, J = 12.0 Hz, Ph*CH*$_2$O), 4.42 (d, 1 H, J = 10.0 Hz, H-1), 4.10 (ddd, 1 H, J = 10.0, 10.0, 8.0 Hz, H-2), 4.09 (s, 2 H, Cl*CH*$_2$CON), 3.93 (d, 1 H, J = 3.2 Hz, H-4), 3.72 (dd, 1 H, J = 10.0, 3.2 Hz, H-3), 3.72-3.64 (m, 3 H, H-5, H-6a and H-6b), and 2.15 (s, 3 H, SMe). ^{13}C NMR ($CDCl_3$): δ = 167.43, 138.30, 137.72, 128.59, 128.54, 127.96, 83.64, 77.58, 75.90, 75.43, 74.28, 73.63, 68.30, 53.23, 42.70, 11.75.

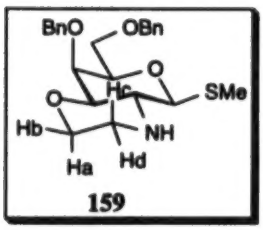
Methyl 4,6-di-O-benzyl-2,3-(3-O-methylenelactam)-2-deoxy-1-thio-β-D-galactopyranoside (**158**).



158

To a solution of compound **157** (140 mg, 300 μmol) in DMF (20 mL), NaH (18 mg, 600 μmol, 80% in oil) was added at 0 °C. The suspension was slowly increased to rt. After the mixture was stirred 4 h, some MeOH was added to the reaction solution to decompose the excess of NaH and then diluted with EtOAc (100 mL). The solution was washed with Brine (3 x 50 mL), dried with $MgSO_4$, filtered, and then concentrated. The resulting residue was subjected to column chromatography (toluene-acetone, 4:1) to give **158** (125 mg, 97%). ^1H NMR ($CDCl_3$): δ = 7.40-7.20 (m, 10 H, aromatics), 6.20 (bs, 1 H, *NH*), 4.88 and 4.57 (2 d, 2 H, J = 11.5 Hz, PhCH_2O), 4.48 and 4.42 (2 d, 2 H, J = 12.0 Hz, PhCH_2O), 4.40 and 4.22 (2 d, 2 H, J = 17.0 Hz, OCH_2CON), 4.20 (d, 1 H, J = 9.8 Hz, H-1), 3.99 (dd, 1 H, J = 2.5, 1.0 Hz, H-4), 3.83 (dd, 1 H, J = 9.8, 9.8 Hz, H-2), 3.75 (ddd, 1 H, J = 7.0, 6.0, 1.0 Hz, H-5), 3.62 (d, 1 H, J = 7.0 Hz, H-6a), 3.62 (d, 1 H, J = 6.0 Hz, H-6b), 3.53 (dd, 1 H, J = 9.8, 2.5 Hz, H-3), and 2.19 (s, 3 H, SMe). ^{13}C NMR ($CDCl_3$): δ = 168.40, 128.48, 128.32, 127.93, 127.86, 127.73, 118.20, 83.96, 79.54, 78.40, 74.47, 73.58, 72.65, 68.48, 68.26, 50.12, 11.35.

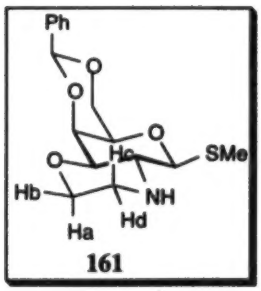
Methyl 2-amino-4,6-di-O-benzyl-2-deoxy-2-N,3-O-(ethane-1,2-diyl)-1-thio-β-D-galactopyranoside (**159**).



159

To a suspension of $LiAlH_4$ (4.7 mg, 117 μmol) in THF (1 mL) was added compound **158** (10 mg, 23 μmol) with vigorous stirring and ice cooling. The reaction mixture was refluxed for 2 h and then cooled in an ice bath. EtOAc was added dropwise slowly and carefully until the vigor of the reaction subsided. The mixture was diluted with EtOAc (20 mL) and filtered through a Celite pad. The residue was washed thoroughly with EtOAc. The combined filtrate was dried with MgSO4, filtered and concentrated. The resulting residue was applied to a silica gel column (toluene-acetone, 3:1 to 1:1) to give product **159** (6.3 mg, 65%; R_f = 0.27, toluene-acetone, 2:1). ^{1}H NMR ($CDCl_3$): δ = 7.40-7.20 (m, 10 H, aromatics), 4.94 and 4.56 (2 d, 2 H, J = 12.0 Hz, Ph*CH*$_2$O), 4.48 and 4.40 (2 d, 2 H, J = 12.0 Hz, Ph*CH*$_2$O), 4.26 (d, 1 H, J = 9.8 Hz, H-1), 3.93 (ddd, 1 H, J = 11.5, 3.5, 1.0 Hz, H-b), 3.79 (dd, 1 H, J = 2.5, 1.0 Hz, H-4), 3.74 (ddd, 1 H, J = 7.0, 5.0, 1.0 Hz, H-5), 3.64 (d, 1 H, J = 11.5, 2.8 Hz, H-a), 3.62 (d, 1 H, J = 7.0 Hz, H-6a), 3.62 (d, 1 H, J = 5.0 Hz, H-6b), 3.40 (dd, 1 H, J = 9.5, 2.5 Hz, H-3), 3.05 (dd, 1 H, J = 9.8, 9.5 Hz, H-2), 3.06 (ddd, 1 H, J = 11.5, 11.5, 3.0 Hz, H-c), 2.90 (dm, 1 H, J = 11.5 Hz, H-d), and 2.19 (s, 3 H, SMe). ^{13}C NMR ($CDCl_3$): δ = 128.41, 128.17, 127.82, 127.36, 85.74, 82.40, 78.26, 74.12, 73.78, 73.52, 69.20, 54.93, 46.34, 12.31. MS(ES): 416.2 $[M+H]^+$ and 438.1 $[M+Na]^+$.

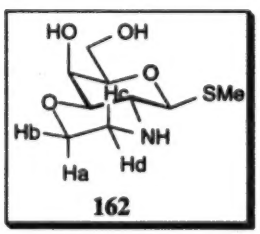
Methyl 2-amino-4,6-O-benzylidene-2-deoxy-2-N,3-O-(ethane-1,2-diyl)-1-thio-β-D-galactopyranoside (**161**).



161

To a suspension of $LiAlH_4$ (400 mg, 10.53 mmol) in THF (200 mL) was added compound **158** (800 mg, 2.37 mmol) with vigorous stirring and ice cooling. The reaction mixture was stirred at rt overnight, refluxed for 2 h and then cooled in an ice bath. EtOAc was added dropwise slowly and carefully until the vigor of the reaction subsided. The mixture was diluted with EtOAc (300 mL) and filtered through a Celite pad. The residue was washed thoroughly with EtOAc. The combined filtrate was washed with satd $NaHCO_3$ and then with water, dried with $MgSO_4$, filtered and concentrated. The resulting residue was applied to a silica gel column (1:1, toluene-acetone containing 1% of Et_3N) to give product **161** (600 mg, 78%; R_f = 0.12, 1:1, toluene-acetone containing 1% of Et_3N). ^1H NMR ($CDCl_3$): δ = 7.55-7.30 (m, 5 H, aromatic), 5.51 (s, 1 H, Ph*CH*O_2), 4.34 (d, 1 H, J = 9.5 Hz, H-1), 4.33 (dd, 1 H, J = 12.5, 1.5 Hz, H-6a), 4.20 (dd, 1 H, J = 3.2, 0.8 Hz, H-4), 4.05 (dd, 1 H, J = 12.5, 1.8 Hz, H-6b), 3.97 (dd, 1 H, J = 11.5, 2.5 Hz, H-b), 3.70 (ddd, 1 H, J = 11.5, 11.5, 1.0 Hz, H-a), 3.55 (d, 1 H, J = 1.5 Hz, H-5), 3.45 (dd, 1 H, J = 9.5, 3.0 Hz, H-3), 3.18 (dd, 1 H, J = 9.5, 9.5 Hz, H-2), 3.10 (ddd, 1 H, J = 11.5, 11.5, 3.0 Hz, H-c), 2.92 (bd, 1 H, J 11.5 Hz, H-d), and 2.25 (s, 3 H, SMe).

Methyl 2-amino-2-deoxy-2-N,3-O-(ethane-1,2-diyl)-1-thio-β-D-galactopyranoside (**162**).

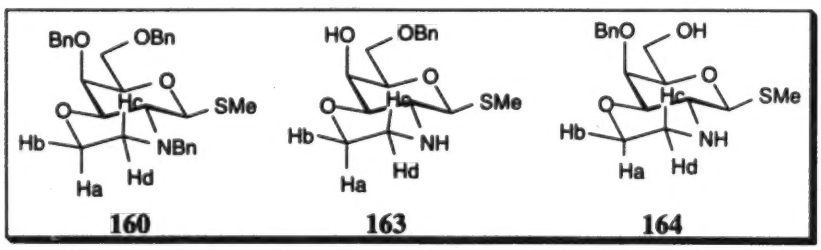


162

A solution of compound **161** (50 mg) in aq HOAc (80%, 10 mL) was heated at 80 °C with stirring for 4 h and then concentrated under vacuum, co-concentrated with toluene (2 x 10 mL). The residue was applied to a silica gel column (20:1, CH_2Cl_2-MeOH containing 1% of Et_3N) to afford **162** (30 mg, 82%; [α] = -54.6°, *c* = 1.2 in $CHCl_3$; R_f = 0.20, toluene-acetone-Et_3N, 1:3:0.3). ^1H

NMR ($CDCl_3$): δ = 4.29 (d, 1 H, J = 9.8 Hz, H-1), 3.99 (dd, 1 H, J = 3.0, 1.0 Hz, H-4), 3.94 (dd, 1 H, J = 12.0, 6.0 Hz, H-6a), 3.97 (ddd, 1 H, J = 11.5, 3.5, 1.0 Hz, H-b), 3.84 (dd, 1 H, J = 12.0, 4.5 Hz, H-6b), 3.72 (ddd, 1 H, J = 11.5, 11.5, 3.0 Hz, H-a), 3.55 (ddd, 1 H, J = 6.5, 4.5, 1.0 Hz, H-5), 3.32 (dd, 1 H, J = 9.8, 3.0 Hz, H-3), 3.04 (ddd, 1 H, J = 11.5, 11.5, 3.5 Hz, H-c), 2.96 (dd, 1 H, J = 9.8, 9.8 Hz, H-2), 2.92 (ddd, 1 H, J = 11.5, 3.0, 1.0 Hz, H-d), and 2.28 (s, 3 H, SMe). ^{13}C NMR ($CDCl_3$): δ = 85.46 (C-1), 80.62, 78.69, 77.48, 68.20, 67.77 (C-6), 62.97 (OCH_2CH_2NH), 54.45, 46.38 CH_2NH), 12.18 (SCH_3).

Methyl 2-amino-2-N-benzyl-4,6-di-O-benzyl-2-deoxy-2-N,3-O-(ethane-1,2-diyl)-1-thio-β-D-galactopyranoside **(160)**, ***Methyl 2-amino-6-O-benzyl-2-deoxy-2-N,3-O-(ethane-1,2-diyl)-1-thio-β-D-galactopyranoside*** **(163)** and ***Methyl 2-amino-4-O-benzyl-2-deoxy-2-N,3-O-(ethane-1,2-diyl)-1-thio-β-D-galactopyranoside*** **(164)**.



To a solution of compound **162** (320 mg, 1.36 mmol) in DMF (15 mL), NaH (123 mg, 4.09 mmol, 80% in oil) was added at 0 °C with stirring. The suspension was slowly increased to rt and then stirred 20 min. BnBr (0.365 mL, 3.0 mmol) was added in dropwise to the reaction solution. After stirring overnight, TLC (**160**, R_f = 0.5, hexane-EtOAc, 2:1; R_f for **159**, **163**, and **164** were 0.63, 0.5 and 0.36, respectively, toluene-acetone-Et_3N, 1:3:0.3) indicated the absence of **162**. Some MeOH was added to the reaction mixture to decompose the excess of NaH and then diluted with EtOAc (300 mL).

The solution was washed with Brine (3 x 150 mL), dried with $MgSO_4$, filtered, and then concentrated. The resulting residue was subjected to column chromatography (4:1 to 1:1, toluene-acetone containing 1% of Et_3N) to give **160** (82 mg, 12%; $[\alpha] = -67.2^o$, $c = 0.1$ in $CHCl_3$), **159** (164 mg, 29%), **164** (23 mg, 5%), and **163** (40 mg, 9%). ^1H NMR ($CDCl_3$):

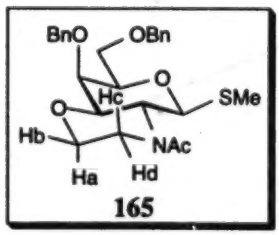
160: δ = 7.50-7.20 (m, 15 H, aromatics), 4.96 and 4.56 (2 d, 2 H, *J* 12.0 Hz, Ph*CH*$_2$O), 4.55 (d, 1 H, J = 9.5 Hz, H-1), 4.48 and 4.41 (2 d, 2 H, J = 12.0 Hz, Ph*CH*$_2$O), 4.11 and 3.72 (2 d, 2 H, J = 12.5 Hz, Ph*CH*$_2$N), 3.84 (dd, 1 H, J = 3.0, 1.0 Hz, H-4), 3.94 (ddd, 1 H, J = 12.0, 12.0, 2.0 Hz, H-a), 3.81 (dd, 1 H, J = 10.0, 3.0 Hz, H-6a), 3.66-3.58 (m, 2 H, H-5 and H-6b), 3.68 (dd, 1 H, J = 9.5, 3.0 Hz, H-3), 3.72 (ddd, 1 H, J = 12.0, 3.5, 1.0 Hz, H-b), 3.46 (dd, 1 H, J = 9.5, 9.5 Hz, H-2), 2.84 (ddd, 1 H, J = 14.0, 12.0, 3.5 Hz, H-c), 2.64 (ddd, 1 H, J = 14.0 2.0, 1.0 Hz, H-d), and 2.25 (s, 3 H, SMe). ^{13}C NMR ($CDCl_3$): δ = 139.10, 138.96, 138.15, 129.75, 129.03, 128.38, 128.29, 128.12, 127.93, 127.74, 127.67, 127.32, 127.13, 84.13, 78.17, 75.45, 74.42, 74.40, 73.49, 69.36, 61.19, 60.19, 50.39, 49.01, 12.74. MS(ES): 506.1 $[M+H]^+$ and 528.2 $[M+Na]^+$.

163: δ = 7.35-7.25 (m, 5 H, aromatic), 4.53 (s, 2 H, Ph*CH*$_2$O), 4.26 (d, 1 H, J = 9.8 Hz, H-1), 3.98 (d, 1 H, J = 2.5 Hz, H-4), 3.92 (ddd, 1 H, J = 11.5, 3.5, 1.0 Hz, H-b), 3.78-3.72 (m, 3 H, H-5, H-6a, and H-6b), 3.71 (ddd, 1 H, J = 11.5, 11.5, 2.5 Hz, H-a), 3.30 (dd, 1 H, J = 9.8, 2.5 Hz, H-3), 3.05 (ddd, 1 H, J = 11.5, 11.5, 3.5 Hz, H-c), 2.95 (dd, 1 H, J = 9.8, 9.8 Hz, H-2), 2.90 (ddd, 1 H, J = 11.5, 2.5, 1.0 Hz, H-d), and 2.20 (s, 3 H, SMe); Acetylated **163**: δ = 7.40-7.20 (m, 5 H, aromatic), 4.55 ang 4.44 (2 d, 2 H, J = 12.0 Hz, Ph*CH*$_2$O), 4.26 (bd, 1 H, J = 10.0 Hz, H-1), 5.44 (dd, 1 H, J = 3.5, 0.8 Hz, H-4), 3.94-3.84 (m, 2 H, H-a and H-b), 3.84 (dd, 1 H, J = 10.0, 3.5 Hz, H-3), 3.69 (sd, 1 H, J = 14.0 Hz, H-d), 3.58 (dd, 1 H, J = 9.5, 6.0 Hz, H-6a), 3.49 (dd, 1 H, J = 9.5, 6.5, Hz, H-6b), 2.59 (ddd, 1 H, J = 11.5, 11.5, 2.5 Hz, H-c), 2.95

(bdd, 2 H, J = 9.5, 9.5 Hz, H-5 and H-2), 2.08 and 2.12 (2 s, 6 H, 2 x Ac), and 2.20 (s, 3 H, SMe).

164: δ = 7.40-7.30 (m, 5 H, aromatic), 4.94 and 4.59 (2 d, 2 H, J = 12.0 Hz, Ph*CH*$_2$O), 4.56 (d, 1 H, J = 9.5 Hz, H-1), 3.94 (ddd, 1 H, J = 11.5, 3.5, 1.0 Hz, H-b), 3.81 (dd, 1 H, J = 11.5, 6.5 Hz, H-6a), 3.70 (dd, 1 H, J = 2.5, 1.0 Hz, H-4), 3.65 (ddd, 1 H, J = 11.5, 11.5, 2.5 Hz, H-a), 3.58 (ddd, 1 H, J = 6.5, 4.5, 1.0 Hz, H-5), 3.49 (dd, 1 H, J = 11.5, 4.5 Hz, H-6b), 3.40 (dd, 1 H, J = 9.5, 2.5 Hz, H-3), 3.07 (ddd, 1 H, J = 11.5, 11.5, 3.5 Hz, H-c), 2.06 (dd, 1 H, J = 9.5, 9.5 Hz, H-2), 2.92 (ddd, 1 H, J = 11.5, 2.5, 1.0 Hz, H-d), and 2.20 (s, 3 H, SMe); Acetylated **164**: δ = 7.40-7.30 (m, 5 H, aromatic), 5.65 (bs, 1 H, H-1), 4.95 ang 4.60 (2 d, 2 H, J = 12.0 Hz, Ph*CH*$_2$O), 4.22 (dd, 1 H, J = 11.5, 6.5 Hz, H-6a), 4.04 (dd, 1 H, J = 11.5, 6.0 Hz, H-6b), 3.83 (dd, 1 H, J = 10.0, 3.0 Hz, H-3), 3.56-3.42 (bs, 1 H, H-2), 1.98 and 2.18 (2 s, 6 H, 2 x Ac), and 2.22 (s, 3 H, SMe).

Methyl 2-acetamido-4,6-di-O-benzyl-2-deoxy-2-N,3-O-(ethane-1,2-diyl)-1-thio-β-D-galactopyranoside (**165**).

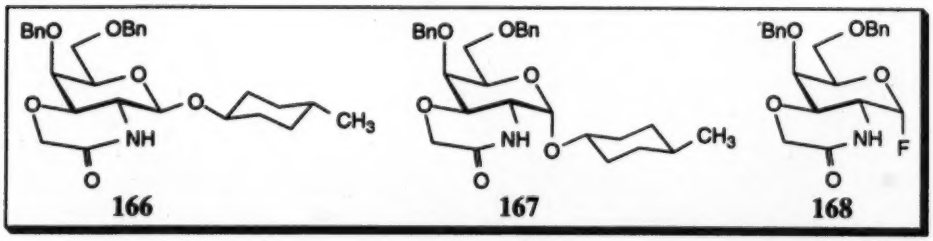


165

A solution of compound **159** (10 mg) in Ac_2O-pyridine (1:2, 1.5 mL) was stirred overnight at rt and then concentrated under vacuum, co-concentrated with toluene (2 x 10 mL). The residue was applied to a silica gel column (toluene-acetone, 3:1) to afforded **165** (quantitative yield; R_f = 0.59, toluene-acetone, 2:1; [α] = -57.4°, *c* = 1.2 in $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ = 7.35-7.20 (m, 10 H, aromatics), 5.52 (bs, 1 H, H-1), 4.91 and 4.54 (2 d, 2 H, J = 11.5 Hz, Ph*CH*$_2$O), 4.46 and 4.38 (2 d, 2 H, J = 12.0 Hz, Ph*CH*$_2$O), 3.84 (d, 1 H, J = 0.8 Hz, H-4), 4.00-3.92 (m, 1 H, H-a), 3.82 (dd, 1 H, J = 13.0, 3.0 Hz, H-b), 3.73 (ddd, 1

H, J = 7.5, 5.0, 0.8 Hz, H-5), 3.72-3.52 (m, 5 H, H-3, 2 x H-6, H-c, H-d), 3.50-3.52 (bs, 1 H, H-2), 2.40 (s, 3 H, NAc), and 2.25 (s, 3 H, SMe). ^{13}C NMR ($CDCl_3$): δ = 171.50, 138.94, 138.07, 128.41, 128.19, 127.91, 127.79, 127.72, 127.43, 77.84, 77.70, 74.67, 74.15, 73.47, 68.89, 66.99, 23.70, 13.32. MS(ES): 480.2 $[M+Na]^+$.

***trans*-4-Methylcyclohexanyl 4,6-di-O-benzyl-2,3-(3-O-methylenelactam)-2-deoxy-β-D-galactopyranoside (166), *trans*-4-Methylcyclohexanyl 4,6-di-O-benzyl-2,3-(3-O-methylenelactam)-2-deoxy-α-D-galactopyranoside (167) and 4,6-di-O-benzyl-2,3-(3-O-methylenelactam)-2-deoxy-β-D-galactopyranosyl fluoride (168).**



A mixture of **158** (16.5 mg, 38.5 μmol), *trans*-4-methylcyclohexanol (19.1 μL, 153.8 μmol) and 4 Å molecular sieves (powder, 250 mg) in dry CH_2Cl_2 (1 mL) was stirred at rt for 1 h and then cooled to -45 °C. To the reaction mixture, $DMTSBF_4$ (15.1 mg, 77 μmol) was added and the solution was increased to -10 °C very slowly in 3 h. TLC showed the absence of starting material. The mixture was filtered through a Celite pad and the Celite pad was washed with CH_2Cl_2. The CH_2Cl_2 solution was washed with Brine (2 x 30 mL), filtered, dried and concentrated. The resulting residue was purified with a silica gel column (toluene-acetone, 4:1) to give **166** (4.4 mg, 23%), **167** (4.0 mg, 19%) and **168** (4.2 mg, 48%). ^1H NMR ($CDCl_3$):

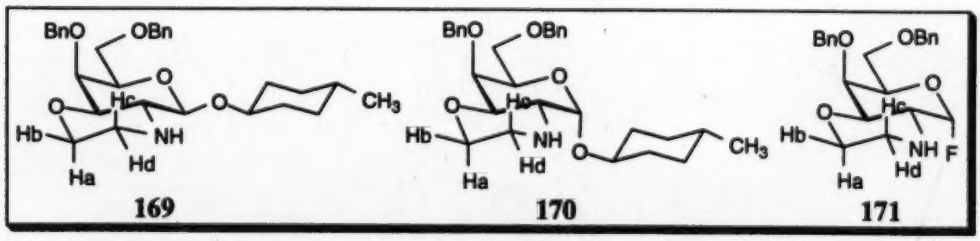
166: δ = 7.35-7.15 (m, 10 H, aromatics), 6.10 (bs, 1 H, *NH*), 4.88 and 4.56 (2 d, 2 H, J = 11.5 Hz, Ph*CH*$_2$O), 4.46 and 4.42 (2 d, 2 H, J = 12.0 Hz, Ph*CH*$_2$O), 4.37 and 4.19 (2 d, 2 H, J = 17.0 Hz, O*CH*$_2$CON), 4.33 (d, 1 H, J = 8.0 Hz, H-1), 3.92 (dd, 1 H, J = 2.5 Hz, H-4), 3.73 (dd, 1 H, J = 10.0, 8.0 Hz, H-2), 3.70-3.52 (m, 4 H, 2 x H-

6, H-5 and O*CH*$(CH_2)_4CHCH_3$), 3.49 (dd, 1 H, J = 10.0, 2.5 Hz, H-3), 2.00-0.90 (m, 9 H, OCH*$(CH_2)_4CH$*CH_3) and 0.85 (d, 3 H, OCH$(CH_2)_4CH$*CH_3*).

Selected ^{1}H NMR ($CDCl_3$) data for **167**: δ = 5.56 (d, 1 H, J = 3.5 Hz, H-1), 4.88 and 4.56 (2 d, 2 H, J = 11.5 Hz, Ph*CH_2*O), 4.51 and 4.40 (2 d, 2 H, J = 12.0 Hz, Ph*CH_2*O), 4.50 and 4.31 (2 d, 2 H, J = 17.0 Hz, O*CH_2*CON), 2.20-0.90 (m, 9 H, OCH*$(CH_2)_4CH$*CH_3) and 0.85 (d, 3 H, OCH$(CH_2)_4CH$*CH_3*).

Selected ^{1}H NMR ($CDCl_3$) data for **168**: δ = 6.27 (dd, 1 H, J = 53.5, 2.2 Hz, H-1), 4.87 and 4.55 (2 d, 2 H, J = 11.5 Hz, Ph*CH_2*O), 4.52 and 4.45 (2 d, 2 H, J = 12.0 Hz, Ph*CH_2*O), 4.45 and 4.35 (2 d, 2 H, J = 16.5 Hz, O*CH_2*CON), 4.13 (ddd, 1 H, J = 24.0, 10.0, 2.2 Hz, H-2).

trans-4-Methylcyclohexanyl 2-amino-4,6-di-O-benzyl-2-deoxy-2-N,3-O-(ethane-1,2-diyl)-β-D-galactopyranoside (**169**), *trans-4-Methylcyclohexanyl 2-amino-4,6-di-O-benzyl-2-deoxy-2-N,3-O-(ethane-1,2-diyl)-α-D-galactopyranoside* (**170**), *2-amino-4,6-di-O-benzyl-2-deoxy-2-N,3-O-(ethane-1,2-diyl)-α-D-galactopyranosyl fluoride* (**171**).



A mixture of **159** (15.0 mg, 36.1 μmol), *trans*-4-methylcyclohexanol (18.0 μL, 144.4 μmol) and 4 Å molecular sieves (powder, 250 mg) in dry CH_2Cl_2 (1 mL) was stirred at rt for 1 h and then cooled to -50 °C. To the reaction mixture, $DMTSBF_4$ (14.3 mg, 72.2 μmol) was added. The solution was increased to 10 °C very slowly in 3 h and then kept overnight. TLC showed the absence of starting material. The mixture was

filtered through a Celite pad and the Celite pad was washed with CH_2Cl_2. The CH_2Cl_2 solution was washed with Brine (2 x 30 mL), filtered, dried and concentrated. The resulting residue was purified with a silica gel column (toluene-acetone, 3:1) to give **169** (4.8 mg, 28%), **170** (6.5 mg, 38%) and **171** (very little < 1 mg). ^1H NMR ($CDCl_3$):

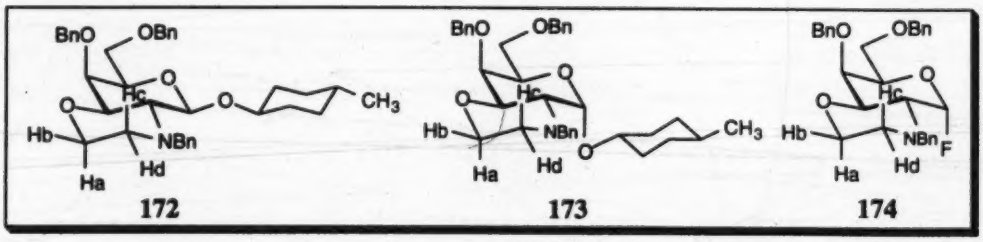
169: δ = 7.40-7.25 (m, 10 H, aromatics), 4.92 and 4.55 (2 d, 2 H, J = 12.0 Hz, Ph*CH*$_2$O), 4.46 and 4.41 (2 d, 2 H, J = 12.0 Hz, Ph*CH*$_2$O), 4.37 (d, 1 H, J = 8.0 Hz, H-1), 3.92 (ddd, 1 H, J = 11.5, 3.0, 1.0 Hz, H-b), 3.73 (dd, 1 H, J = 2.5, 0.8 Hz, H-4), 3.72-3.50 (m, 6 H, 2 x H-6, H-5, H-a, H-b and O*CH*$(CH_2)_4CHCH_3$), 3.42 (dd, 1 H, J = 10.0, 2.5 Hz, H-3), 3.04 (ddd, 1 H, J = 11.5, 11.5, 3.5 Hz, H-c), 3.0 (dd, 1 H, J = 10.0, 8.0 Hz, H-2), 2.88 (bd, 1 H, J = 11.5 Hz, H-d), 2.20-0.90 (m, 9 H, OCH*(CH$_2$)$_4$CH*CH$_3$) and 0.85 (d, 3 H, OCH$(CH_2)_4$CH*CH$_3$*).

170: δ = 7.35-7.20 (m, 10 H, aromatics), 4.95 (d, 1 H, J = 3.8 Hz, H-1), 4.94 and 4.54 (2 d, 2 H, J = 11.5 Hz, Ph*CH*$_2$O), 4.49 and 4.40 (2 d, 2 H, J = 12.0 Hz, Ph*CH*$_2$O), 4.08 (td, 1 H, J = 6.5, 0.8 Hz, H-5), 3.91 (dd, 1 H, J = 11.5, 3.2 Hz, H-b), 3.78 (dd, 1 H, J = 2.5, 0.8 Hz, H-4), 3.62 (dd, 1 H, J = 10.0, 2.5 Hz, H-3), 3.58-3.43 (m, 4 H, 2 x H-6, H-a, and O*CH*$(CH_2)_4CHCH_3$), 3.28 (dd, 1 H, J = 10.0, 3.8 Hz, H-2), 3.04 (ddd, 1 H, J = 14.0, 11.5, 3.5 Hz, H-c), 2.91 (dd, 1 H, J = 14.0, 2.0 Hz, H-d), 2.00-0.90 (m, 9 H, OCH*(CH$_2$)$_4$CH*CH$_3$) and 0.85 (d, 3 H, OCH$(CH_2)_4$CH*CH$_3$*).

Selected ^1H NMR ($CDCl_3$) data for **171**: δ = 6.18 (dd, 1 H, J = 52.2, 2.2 Hz, H-1), 4.89 and 4.52 (2 d, 2 H, J = 11.5 Hz, Ph*CH*$_2$O), 4.49 and 4.41 (2 d, 2 H, J = 12.0 Hz, Ph*CH*$_2$O), 3.74 (ddd, 1 H, J = 11.5, 11.5, 2.5 Hz, H-a), 3.16 (ddd, 1 H, J = 25.0, 10.0, 2.2 Hz, H-2) and 2.97 (ddd, 1 H, J = 11.5, 2.5, 1.0 Hz, H-d).

trans-4-Methylcyclohexanyl 2-amino-2-N-benzyl-4,6-di-O-benzyl-2-deoxy-2-N,3-O-(ethane-1,2-diyl)-β-D-galactopyranoside (**172**), *trans-4-Methylcyclohexanyl 2-amino-2-*

N-benzyl-4,6-di-O-benzyl-2-deoxy-2-N,3-O-(ethane-1,2-diyl)-α-D-galactopyranoside (**173**), *2-amino-2-N-benzyl-4,6-di-O-benzyl-2-deoxy-2-N,3-O-(ethane-1,2-diyl)-β-D-galactopyranosyl fluoride* (**174**).



172 **173** **174**

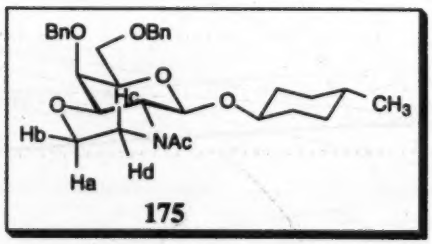
A mixture of **160** (10.0 mg, 19.8 μmol), *trans*-4-methylcyclohexanol (15.0 μL, 188.1 μmol) and 4 Å molecular sieves (powder, 200 mg) in dry CH_2Cl_2 (0.8 mL) was stirred at rt for 1 h and then cooled to -45 °C. To the reaction mixture, $DMTSBF_4$ (8.0 mg, 39.6 μmol) was added. The solution was increased to -10 °C very slowly and then kept for 10 h. TLC showed the absence of starting material. The mixture was filtered through a Celite pad and the Celite pad was washed with CH_2Cl_2. The CH_2Cl_2 solution was washed with Brine (2 x 30 mL), filtered, dried and concentrated. The resulting residue was purified with a silica gel column (toluene-acetone, 4:1) to give **172** (0.8 mg, 7.1%), **173** (5.0 mg, 44%) and **174** (3.0 mg, 33%). ^1H NMR ($CDCl_3$):

172: δ = 7.40-7.20 (m, 15 H, aromatics), 4.90 and 4.58 (2 d, 2 H, J = 12.0 Hz, Ph*CH*$_2$O), 4.48 and 4.42 (2 d, 2 H, J = 12.0 Hz, Ph*CH*$_2$O), 4.60 (d, 1 H, J = 7.5 Hz, H-1), 4.91 and 3.30 (2 d, 2 H, J = 13.5 Hz, Ph*CH*$_2$N), 3.79 (bdd, 1 H, J = 11.5, 2.0 Hz, H-b), 3.74 (bs, 1 H, H-4), 3.67-3.52 (m, 4 H, 2 x H-6, H-a, and O*CH*$(CH_2)_4CHCH_3$), 3.45 (dd, 1 H, J = 10.0, 2.0 Hz, H-3), 2.85 (dd, 1 H, J = 10.0, 7.5 Hz, H-2), 2.59 (bd, 1 H, J = 12.0 Hz, H-d), 2.39 (ddd, 1 H, J = 11.5, 11.5, 3.5 Hz, H-c), 2.10-0.85 (m, 9 H, OCH*$(CH_2)_4CH$*CH_3) and 0.83 (d, 3 H, OCH$(CH_2)_4CH$*CH_3*).

173: δ = 7.40-7.20 (m, 15 H, aromatics), 5.39 (d, 1 H, J = 3.5 Hz, H-1), 4.94 and 4.55 (2 d, 2 H, J = 12.0 Hz, Ph*CH*$_2$O), 4.51 and 4.43 (2 d, 2 H, J = 12.0 Hz, Ph*CH*$_2$O), 4.14 (td, 1 H, J = 6.2, 1.0 Hz, H-5), 4.08 and 3.00 (2 d, 2 H, J = 12.5 Hz, Ph*CH*$_2$N), 3.91 (dd, 1 H, J = 10.0, 2.8 Hz, H-3), 3.74 (dd, 1 H, J = 2.5, 0.8 Hz, H-4), 3.76 (ddd, 1 H, J = 11.5, 3.5, 1.0 Hz, H-b), 3.68 (ddd, 1 H, J = 11.5, 11.5, 2.3 Hz, H-a), 3.63-3.52 (m, 3 H, 2 x H-6 and O*CH*(CH$_2$)$_4$CHCH$_3$), 2.82 (dd, 1 H, J = 10.0, 3.5 Hz, H-2), 2.55 (bd, 1 H, J = 11.5 Hz, H-d), 2.19 (ddd, 1 H, J = 11.5, 11.5, 3.5 Hz, H-c), 2.05-0.90 (m, 9 H, OCH(*CH$_2$)$_4$CH*CH$_3$) and 0.85 (d, 3 H, OCH(CH$_2$)$_4$CH*CH$_3$*).

174: δ = 7.35-7.20 (m, 15 H, aromatics), 6.00 (dd, 1 H, J = 53.0, 2.2 Hz, H-1), 4.52 and 4.55 (2 d, 2 H, J = 11.5 Hz, Ph*CH*$_2$O), 4.52 and 4.44 (2 d, 2 H, J = 12.0 Hz, Ph*CH*$_2$O), 4.14 and 3.13 (2 d, 2 H, J = 12.8 Hz, Ph*CH*$_2$N), 4.23 (bt, 1 H, J = 6.5 Hz, H-4), 3.91 (m, 1 H, H-3), 3.83 (ddd, 1 H, J = 11.5, 3.5, 1.0 Hz, H-b), 3.72-3.55 (m, 4 H, 2 x H-6, H-a, and H-5), 2.89 (ddd, 1 H, J = 25.0, 10.0, 2.2 Hz, H-2), 2.64 (bd, 1 H, J = 11.5 Hz, H-d), 2.39 (dddd, 1 H, J = 11.5, 11.5, 3.5, 1.5 Hz, H-c).

trans-4-Methylcyclohexanyl 2-amino-2-N-acetyl-4,6-di-O-benzyl-2-deoxy-2-N,3-O-(ethane-1,2-diyl)-β-D-galactopyranoside (**175**).

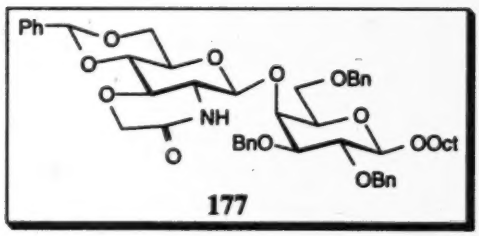


175

A mixture of **165** (15.0 mg, 32.8 μmol), *trans*-4-methylcyclohexanol (25.0 μL, 196.8 μmol) and 4 Å molecular sieves (powder, 200 mg) in dry CH_2Cl_2 (0.8 mL) was stirred at rt for 1 h and then cooled to -45 °C. To the reaction mixture, $DMTSBF_4$ (40.0 mg, 196.8 μmol) was added. The solution was increased to 0 °C very slowly. TLC showed the absence of starting material and a new spot for intermediate **176** and all **176** was converted into **175** after 10 h. The mixture was filtered

through a Celite pad which was washed with CH_2Cl_2. The CH_2Cl_2 solution was washed with Brine (2 x 30 mL), filtered, dried and concentrated. The resulting residue was purified with a silica gel column (toluene-acetone, 4:1) to give **175** (14.5 mg, 85%). ^1H NMR ($CDCl_3$): δ = 7.35-7.20 (m, 10 H, aromatics), 5.14-5.04 (bs, 1 H, H-1), 4.87 and 4.53 (2 d, 2 H, J = 11.5 Hz, Ph*CH*$_2$O), 4.45 and 4.39 (2 d, 2 H, J = 12.0 Hz, Ph*CH*$_2$O), 4.10-4.00 (bs, 1 H, H-2), 3.95 (m, 1 H, H-a), 3.82 (bs, 1 H, H-4), 3.74 (m, 2 H, H-a and H-3), 3.66 (ddd, 1 H, J = 7.5, 6.0, 1.0 Hz, H-5), 3.54-3.48 (m, 4 H, 2 x H-6, H-c, and O*CH*$(CH_2)_4$CHCH$_3$), 3.41-3.30 (m, 1 H, H-d), 2.20 (s, 3 H, NAc), 2.10-0.85 (m, 9 H, OCH(*CH*$_2)_4$*CH*CH$_3$) and 0.83 (d, 3 H, OCH$(CH_2)_4$CH*CH*$_3$).

Octyl 4-O-[4,6-O-benzylidene-2,3-(3-O-methylenelactam)-2-deoxy-β-D-glucopyranosyl]-2,3,6-tri-O-benzyl-β-D-galactopyranoside (**177**).

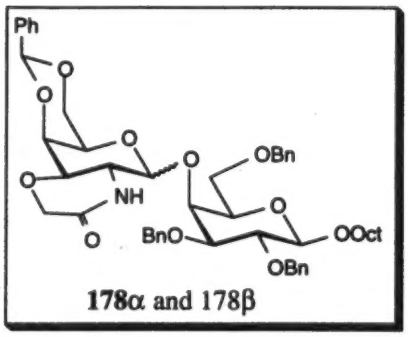


177

A mixture of **150** (10.0 mg, 25 μmol), **54** (14.1 mg, 25 μmol), NIS (8.5 mg, 37.5 μmol) and dried 4 Å molecular sieves (powder, 100 mg) in CH_2Cl_2 (1 mL) was stirred at rt for 30 min and then cooled to -60 °C. To this mixture was added TfOH (0.44 μL, 5 μmol) and the temperature was increased to 0 °C slowly. Stirring was continued at 0 °C for 3 h before Et_3N was added to quench the reaction. The mixture was filtered through Celite and the filtrate was diluted with CH_2Cl_2, washed with Brine, dried with $MgSO_4$, filtered and concentrated in vacuo. The resulting residue was chromatographed on a silica gel column (hexane-ethyl acetate, 3:1) to give **177** (low yield). Selected ^1H NMR ($CDCl_3$) data: δ = 5.51 (s, 1 H, Ph*CH*O_2), 4.96 and 4.69 (2 d, 2 H, J = 12.0 Hz, Ph*CH*$_2$O), 4.96 and 4.78 (2 d, 2 H, J =

11.0 Hz, PhCH_2O), 4.49 (s, 2 H, PhCH_2O), 4,42 (d, 1 H, J = 8.0 Hz, H-1'), 4.34 (d, 1 H, J = 7.5 Hz, H-1), 4.34 and 4.19 (2 d, 2 H, J = 17.0 Hz, OCH_2CON).

Octyl 4-O-[4,6-O-benzylidene-2,3-(3-O-methylenelactam)-2-deoxy-α-D-galactopyranosyl]-2,3,6-tri-O-benzyl-β-D-galactopyranoside (**178α**) and *Octyl 4-O-[4,6-O-benzylidene-2,3-(3-O-methylenelactam)-2-deoxy-β-D-galactopyranosyl]-2,3,6-tri-O-benzyl-β-D-galactopyranoside* (**178β**).



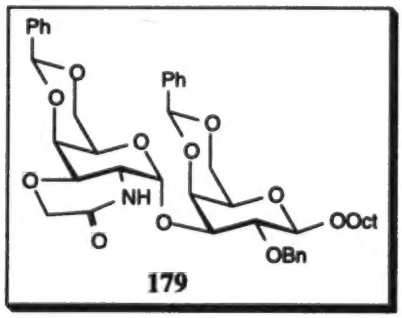
178α and 178β

A mixture of **144** (9.0 mg, 25.5 μmol), **54** (11.5 mg, 20.0 μmol), NIS (11.5 mg, 51.0 μmol) and dried WA-300 molecular sieves (powder, 100 mg) in CH_2Cl_2 (1 mL) was stirred at rt for 30 min and then cooled to -78 °C. To this mixture was added TfOH (0.23 μL, 2.6 μmol) and the temperature was increased to 10 °C slowly. Stirring was continued at 10 °C for 10 h before Et_3N was added to quench the reaction. The mixture was filtered through Celite and the filtrate was diluted with CH_2Cl_2, washed with Brine, dried with $MgSO_4$, filtered and concentrated in vacuo. The resulting residue was chromatographed on a silica gel column (hexane-EtOAc, 3:1) to give **178α** and **178β** (2:1, low yield < 20%).

Selected ^{1}H NMR ($CDCl_3$) data for **178α**: δ = 6.36 (s, 1 H, NH), 5.58 (s, 1 H, PhCHO_2), 5.15 (d, 1 H, J = 3.5 Hz, H-1'), 5.03 and 4.79 (2 d, 2 H, J = 11.0 Hz, PhCH_2O), 4.97 and 4.55 (2 d, 2 H, J = 11.0 Hz, PhCH_2O), 4.93 and 4.68 (2 d, 2 H, J = 12.0 Hz, PhCH_2O), 4.42 (d, 1 H, J = 7.5 Hz, H-1).

Selected ^{1}H NMR ($CDCl_3$) data for **178β**: δ = 6.66 (s, 1 H, NH), 5.58 (s, 1 H, Ph*CH*O_2), 4.80 (d, 1 H, J = 8.0 Hz, H-1'), 5.07 and 4.58 (2 d, 2 H, J = 11.0 Hz, Ph*CH*$_2$O), 4.42 (d, 1 H, J = 7.0 Hz, H-1).

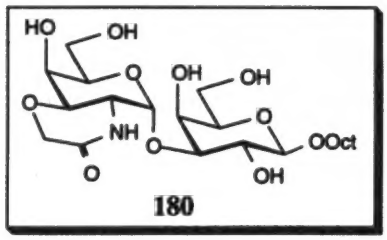
Octyl 3-O-[4,6-O-benzylidene-2,3-(3-O-methylenelactam)-2-deoxy-α-D-galactopyranosyl]-4,6-O-benzylidene-β-D-galactopyranoside (**179**).



179

A mixture of **144** (19.0 mg, 54.0 μmol), **42** (20.2 mg, 43.0 μmol), NIS (24.3 mg, 108.0 μmol) and dried WA-300 molecular sieves (powder, 200 mg) in CH_2Cl_2 (1.5 mL) was stirred at rt for 30 min and then cooled to -78 °C. To this mixture was added TfOH (0.5 μL, 5.4 μmol) and the temperature was increased to 10 °C slowly. Stirring was continued at 10 °C for 10 h before Et_3N was added to quench the reaction. The mixture was filtered through Celite and the filtrate was diluted with CH_2Cl_2, washed with Brine, dried with $MgSO_4$, filtered and concentrated in vacuo. The resulting residue was chromatographed on a silica gel column (hexane-ethyl acetate, 2:1) to give **179** (12 mg, 36%). Selected ^{1}H NMR ($CDCl_3$) data: δ = 5.58 (s, 1 H, *NH*), 5.56 (s, 1 H, Ph*CH*O_2), 5.44 (s, 1 H, Ph*CH*O_2), 5.12 (d, 1 H, J = 3.5 Hz, H-1'), 5.02 and 4.55 (2 d, 2 H, J = 11.5 Hz, Ph*CH*$_2$O), 4.41 (d, 1 H, J = 7.5 Hz, H-1), 4.37 and 4.29 (2 d, 2 h, J = 13.0 Hz, O*CH*$_2$CON).

Octyl 3-O-[2,3-(3-O-methylenelactam)-2-deoxy-α-D-galactopyranosyl]-β-D-galactopyranoside (**180**).



180

Hydrogenolysis of **179** (6 mg) was performed in MeOH (5 mL) with $Pd(OH)_2/C$ (10 mg) at rt for 8 h. The reaction mixture was filtered through a Millex-GV filter unit and concentrated in vacuo to give **180** in quantitative yield. Selected ^1H NMR (CD_3OD) data: δ = 5.18 (d, 1 H, J = 3.5 Hz, H-1'), 4.26 (d, 1 H, J = 7.5 Hz, H-1) and 4.24 (s, 2 H, OCH_2CON).

Chapter 7

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